

This Page Is Inserted by IFW Operations  
and is not a part of the Official Record

## **BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning documents *will not* correct images,  
please do not report the images to the  
Image Problem Mailbox.**

**THIS PAGE BLANK (USPTO)**

(19)



Europäisches Patentamt  
European Patent Office  
Office européen des brevets



(11) Publication number:

**0 564 924 A2**

(12)

**EUROPEAN PATENT APPLICATION**(21) Application number: **93105035.5**(22) Date of filing: **26.03.93**

(51) Int. Cl.<sup>5</sup>: **C07K 5/06**, C07D 207/16,  
C07D 401/12, C07D 405/12,  
C07D 401/06, C07D 211/60,  
A61K 37/02, A61K 31/40,  
A61K 31/44, A61K 31/445

(30) Priority: **08.04.92 US 864998**  
**25.11.92 US 981565**

(43) Date of publication of application:  
**13.10.93 Bulletin 93/41**

(84) Designated Contracting States:  
**AT BE CH DE DK ES FR GB GR IE IT LI LU MC**  
**NL PT SE**

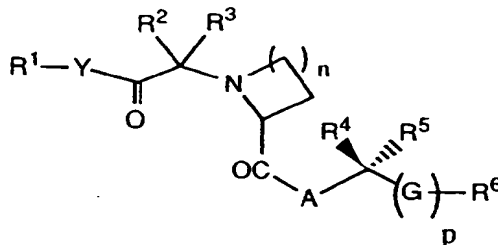
(71) Applicant: **MILES INC.**  
**One Mellon Center**  
**500 Grant Str.**  
**Pittsburgh, PA 15219-2502(US)**

(72) Inventor: **Connell, Richard D.**  
**3 Raynham Road**  
**New Haven, CT 06512(US)**  
Inventor: **Osterman, David D.**  
**715 Manchester Road**  
**Glastonbury, CT 06033(US)**  
Inventor: **Katz, Michael E.**  
**6 Nod Brook Road**  
**Wallingford, CT 06492(US)**

(74) Representative: **Dänner, Klaus, Dr. et al**  
**Bayer AG**  
**Konzernverwaltung RP**  
**Patente Konzern**  
**D-51368 Leverkusen (DE)**

(54) **2-Oxoethyl derivatives as immunosuppressants.**

(57) A class of compounds which suppress human T-lymphocyte proliferation is disclosed. The active compounds essentially contain at least the following structure:

**EP 0 564 924 A2**

## BACKGROUND

The invention relates to methods and compounds for controlling inflammatory processes in humans through mediation of inflammatory cell proliferation. More particularly, the present invention is a method for suppressing T-lymphocytes using a class of novel compounds which bind to the FKBP-type family of immunophilins.

Compounds which retard the production of cytokines such as interleukin-2 (IL-2) are known. For instance, U.S. Patent No. 4,764,503 assigned to Sandoz Ltd., Basel, Switzerland, describes a compound generically referred to as Cyclosporin A (hereinafter referred to as "CsA"), and U.S. Patent No. 4,894,366 assigned to Fujisawa Pharmaceuticals, Osaka, Japan, describes a compound they designate as "FK506." Both CsA and FK 506 are claimed to inhibit IL-2 production and bind to cellular receptor proteins that possess Peptidyl Prolyl Isomerase (PPIase) activity (Johansson et al., 1990, Transplantation 50:10017).

It was initially postulated by those skilled in the art that the specific binding by such compounds to PPIase type proteins led to inhibition of the protein's isomerase activity which, in turn, led to inhibition of T-cell proliferation. Thus, these PPIase type proteins were referred to as "immunophilins", with the cellular receptor proteins that bound to CsA and FK506 being referred to as "cyclophilin" and "FK506 binding protein" respectively. FK506 binding protein is also simply referred to as "FKBP" (Harding et al., 1989, Nature 341:758).

Recent publications report that the inhibition of PPIase activity, in and of itself, is not sufficient for immunosuppressant activity. However, there is support in the literature that inhibitory binding to PPIase-type enzymes probably contributes to ultimate T-cell suppression (Sigal et al. 1991, J. Exp. Med. 173:619).

This disclosure presents a new class of synthetic compounds that both suppress the proliferation of T-cells and inhibit the isomerase activity of the FKBP-type of PPIases.

CsA, a cyclic undecapeptide, has received FDA approval for use as an adjunct to organ transplant procedures. However, CsA is administered with caution due to its known toxicity. Currently, CsA is prescribed in situations where the risks of non treatment outweigh the risks of its therapeutic complications.

As a result, efforts to expand the application of CsA into non life threatening indications such as chronic maintenance of autoimmune disorders have been limited by the well-known side effects of this drug. The use of CsA leads to a variety of disorders including: nephrotoxicity, such as impairment of glomerular filtration and irreversible interstitial fibrosis (Kopp et al., 1991, J. Am. Soc. Nephrol. 1:162); neurological deficits, such as involuntary tremors, or non-specific cerebral angina such as non-localized headaches (De Groen et al, 1987, N. Engl. J. Med. 317:861); and vascular hypertension with complications resulting therefrom (Kahan et al., 1989, N. Engl. J. Med. 321:1725).

Recent efforts to investigate the cause of the adverse effects of CsA administration have centered on the role of CsA breakdown into toxic metabolites (Bowers et al., 1990, Clin. Chem. 36:1875; Burke et al., 1990, Transplantation 50:901). The prevailing thought is that CsA toxicity is due to such metabolites and not due to the nature of the CsA binding to the PPIase, cyclophilin (Akagi et al., 1991, J. Int. Med. Res. 19:1; Ryffel et al., 1988, Transplantation 46:905).

Thus, inhibitor compounds that do not resemble CsA structurally, yet bind to PPIases, should be more amenable to therapeutic applications. Such non-toxic immunosuppressors would benefit the art, especially for chronic administration such as required in the treatment of autoimmune disorders.

The compound FK506 is structurally different from CsA and does not produce the same type of toxic metabolites. FK506 has been shown to be effective in some transplant patients who do not respond to CsA (Tucci et al., 1989, J. Immunol. 143:718).

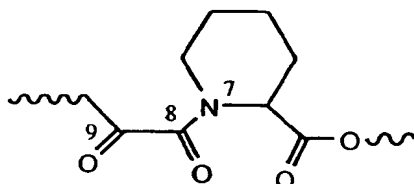
However, testing of FK506 in humans was delayed due to severe vasculitis observed in treatment regimens in dogs and baboons (Collier et al., 1988, Transplant Proc. 20:226). Furthermore, other clinical side effects and complications of FK506 administration are being reported (Frayha et al., 1991, Lancet 337:296; Kitahara et al., 1991, Lancet 337:1234). It has also been reported that "overall, the absolute rate of clinical rejection in FK506 [post-organ transplantation] patients is only slightly lower than with current standard therapies" (Holechek, 1991, Anna. J. 18:199).

In an attempt to alleviate the FK506 side effects, many minor modifications to the base structure have been reported. For example, U.S. Patent No. 5,057,608 assigned to Merck & Co. and WIPO Publication No. WO89/05304 assigned to Fisons PLC Inc. both disclose chemical variations of the FK506 compound.

To date only a few studies on the metabolism of FK506 have been published, and little information has been reported on the toxicity of its metabolites (Johansson et al., 1990, Transplantation 50:1001; Christians et al., 1991, Clinical Biochemistry 24:271; Lhoest et al., 1991, Pharmaceutica Acta Helveticae 66:302). Since it is likely that the pattern of metabolism of the FK506 analogs and derivatives are similar to the parent compound, it is also likely that many of the side effects of FK506 will be shared by the derivatives.

As is true for CsA, the toxicity of FK506 is postulated to be based on its structure and not due to its binding activity with the immunophilin FKBP. It is further postulated that the toxicity of compounds such as CsA and FK506 are due to various chemical groups found in these structures which do not participate in the immunosuppressive activity, such as those groups which result in the toxic metabolites of CsA bioprocessing. Thus, relatively compact molecules which do not resemble either CsA or FK506, and which have both immuno-suppressive and PPlase binding activity should be free of side effects associated with CsA and FK506.

Furthermore, the compound FK506 and its derivatives (for example such as disclosed in WIPO Publication No. WO92/00278 assigned to VERTEX Pharmaceuticals Inc.) all share the following homoproline (6-membered, proline-like) dicarbonyl backbone structure:



(I)

FK506 and its derivatives all preferably rely on the two carbonyl groups at positions 8 and 9, with the presence of the carbonyl at the number 8 carbon being essential. The presence of the double bond oxygen in proximity to number 7 nitrogen creates an amide type linkage between the nitrogen at position 7 and carbon at position 8.

Recent reports have suggested that the nitrogen at position 7, along with the number 8 and 9 carbonyl groups of FK506 represent "a twist-bond amide" (Michnick et al., 1991, Science 252:836). Based on the data presented in the Michnick et al. article, it was assumed and accepted by those skilled in this art that the carbonyl at position 8 was the functional species. Jorgensen, 1991, Science 254:954, teaches that this keto-amide moiety is critical to activity because the moiety allegedly serves as a transition state analog.

The present description proposes that the carbonyl group at the number 8 position is non-essential for T-cell suppression, and the compounds of the present invention do not rely on this carbonyl group.

The present invention presents a novel class of synthetic inhibitor compounds. The novel class includes synthetic 2-oxoethylene derivatives that bind to human FKBP-type PPlases and demonstrate human peripheral T-lymphocyte inhibitory activity. Moreover, the absence of a carbonyl attached directly to the nitrogen in the proline ring (see formula II, below) provides compounds that possess stability to hydrolysis by proteases at the N-terminus of proline.

It is therefore an object of the present invention to provide for compounds and compositions containing such 2-oxo-ethylene derivatives for suppression of pathological and abnormal human peripheral T-lymphocyte proliferation.

It is also an object of the present invention to provide a novel class of compounds suitable for therapeutic compositions designed to suppress pathological immune responses, such as the hyperimmune response in organ transplantation rejection, the self-destructive autoimmune diseases, and the overproduction and excessive proliferation of immune cells such as in infectious disease states.

More specific objects include provisions for compounds, compositions and methods for treatment and prevention of rejection of transplanted organs or tissues such as kidney, heart, lung, liver, bone marrow, skin grafts, and corneal replacement.

It is a further object to provide compounds, compositions and methods for use in the treatment of autoimmune, degenerative, inflammatory, proliferative and hyperproliferative diseases, such as rheumatoid arthritis, osteoarthritis, other degenerative joint diseases, joint inflammation such as associated with infectious diseases such as suppurative arthritis, and secondary arthritis such as those associated with gout, hemochromatosis, rheumatic fever, Sjögrens syndrome and tuberculosis.

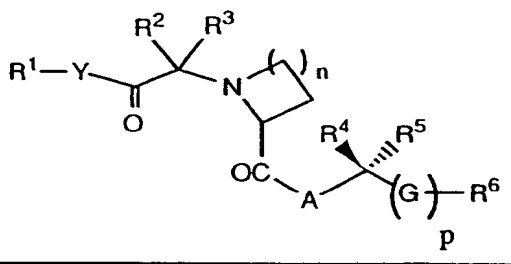
Another object is to provide compounds, compositions and methods for use in the treatment of lupus erythematosus, systemic lupus erythematosus, Hashimoto's thyroiditis, multiple sclerosis, myasthenia gravis, type 1 diabetes, uveitis, nephrotic syndrome, and of cutaneous manifestations of immunologically-mediated diseases such as psoriasis, atopic dermatitis, contact dermatitis, eczematous dermatitides, seborrheic dermatitis, lichen planus, pemphigus, bullous pemphigoid, epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, and alopecia areata.

Yet another object is to provide compounds, compositions and methods for use in the treatment of abnormal T-cell proliferation such as lymphocytic leukemia; Hodgkin's disease, especially those subtypes involving abnormal T-cell subpopulations; non-Hodgkin's lymphomas, such as mycosis fungoides, convulated lymphocytic lymphoma, and immunoblastic sarcoma; and chronic lymphadenitis.

The above lists are non-limiting, and one skilled in the art could easily adapt the compounds, compositions and methods of the present invention to other indications, such adaptations being within the spirit and scope of the invention which will be described hereinbelow.

# SUMMARY OF THE INVENTION

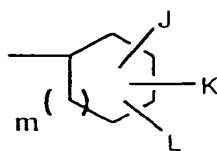
The presently claimed invention relates to compounds comprising at least one of the following structures:



where

R<sup>1</sup> is

- a) hydrogen,
- b) linear or branched alkyl (C1-C8) which may be substituted independently or simultaneously up to two times by
  - i) hydroxy,
  - ii) phenyl which may be substituted by straight or branched alkyl (C1-C8), or straight or branched alkoxy (C1-C6),
  - iii) cycloalkyl (C3-C10) which may be substituted by straight or branched alkyl (C1-C8), or straight or branched alkoxy (C1-C6),
  - iv) bicycloalkyl (C6-C12) which may be substituted by straight or branched alkyl (C1-C10), or straight or branched alkoxy (C1-C6),
  - v) tricycloalkyl (C7-C14) which may be substituted by straight or branched alkyl (C1-C8), or straight or branched alkoxy (C1-C6),
  - vi) tetracycloalkyl (C10-C14), which may be substituted by straight or branched alkyl (C1-C8), or straight or branched alkoxy (C1-C6),
  - vii) morpholinyl.
- c) alkene (C3-C10), diene (C4-C10), or triene (C8-C18), which may be substituted independently or simultaneously up to three times by
  - i) phenyl,
  - ii) straight or branched alkyl (C1-C6),
  - iii) straight or branched alkoxy (C1-C6),
- d) cycloalkyl (C5-C10), or the cycloalkyl fragment

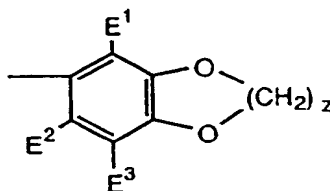


where

m is an integer of 0, 1, or 2;

J, K, and L are independently or simultaneously

- i) hydrogen
- ii) straight or branched alkyl (C1-C5), which may be substituted by phenyl, or straight or branched alkoxy (C1-C6).
- 5 iii) straight or branched alkoxy (C1-C5)
- iii) phenyl, or
- iv) phenyl substituted by straight or branched alkyl (C1-C6), or chlorine, or straight or branched alkoxy (C1-C6).
- e) bicycloalkyl (C7-10), tricycloalkyl (C7-14), tetracycloalkyl (C10-C16), or pentacycloalkyl (C11-C20),
- 10 which may be independently or simultaneously substituted up to 3 times with straight or branched alkyl (C1-C6), or straight or branched alkoxy (C1-C6), or phenyl
- f) the aryl derivatives tetrahydronaphthyl, benzothienyl, benzofuryl, benzopyranyl, furyl, pyridyl, pyranyl, 1,3-oxazolyl, or naphthyl. These aryl derivatives may be independently or simultaneously substituted up to two times by
- 15 i) straight or branched alkyl (C1-C6),
- ii) straight or branched alkoxy (C1-C6)
- iii) halogen, where halogen is fluoro, chloro, bromo, or iodo.
- g) the piperonyl fragment

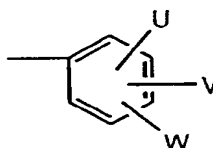


where

z is an integer of 1, or 2;

and E¹, E², and E³ can be independently or simultaneously hydrogen, straight or branched alkyl (C1-C6), straight or branched alkoxy (C1-C6), or chlorine.

h) the aryl derivative



where

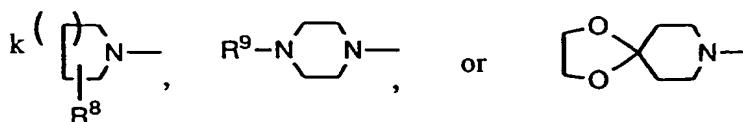
U, V, and W can be independently or simultaneously

- i) hydrogen
- ii) straight or branched alkyl (C1-C6), straight or branched alkoxy (C1-C6), phenyl, or phenoxy. These
- 45 group may be substituted by phenyl, straight or branched alkoxy (C1-C6), or phenoxy.
- iii) hydroxy,
- iv) halogen
- v) nitro,
- vi) benzoyl,

Y is a covalent bond, oxygen, NR⁷, where R⁷ is hydrogen.

in addition to that described above,

R¹—Y— may also be



where

k is an integer of 1, or 2;

R<sup>8</sup> is

a) hydrogen.

b) carboalkoxy with a straight or branched alkoxy (C1-C6)

c) straight or branched alkyl (C1-C6) which may be substituted by phenyl, or straight or branched alkoxy (C1-C6)

d) phenyl, or phenyl substituted by halogen

R<sup>9</sup> is phenyl which may be substituted by straight or branched alkyl (C1-C6).

R<sup>2</sup> and R<sup>3</sup> are defined as follows: one of R<sup>2</sup> and R<sup>3</sup> are hydrogen, and the other is hydrogen or straight or branched alkyl (C1-C6).

n is an integer of 2 or 3.

A is oxygen or NR<sup>10</sup>, where R<sup>10</sup> is hydrogen or straight or branched alkyl (C1-C6).

R<sup>4</sup> and R<sup>5</sup> may independently or simultaneously be

a) hydrogen.

b) straight or branched alkyl (C1-C8) which may be substituted by

i) phenyl, or phenyl substituted by hydroxy or alkoxy (C1-C2)

ii) cycloalkyl (C5-C6)

iii) alkylthio (C1-C6)

iv) carboxamido

v) straight or branched alkoxy (C1-C6) which may be substituted by phenyl

c) phenyl

d) cycloalkyl (C3-C7), which may be substituted by straight or branched alkyl (C1-C6)

In addition, R<sup>4</sup> and R<sup>5</sup>, taken together can be

-(CH<sub>2</sub>)<sub>r</sub>-

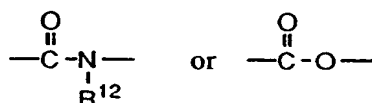
where

r is an integer of 4 or 5.

G is one of the fragments

-HC=CH-, -CH<sub>2</sub>•CH<sub>2</sub>-, or -CH<sub>2</sub>-

or one of the fragments



where R<sup>12</sup> is hydrogen or methyl, and it is understood that the carbonyl group is attached to the carbon bearing R<sup>4</sup> and R<sup>5</sup> and that NR<sup>12</sup> and oxygen are connected to R<sup>6</sup>, respectively,

p is an integer of 0 or 1.

R<sup>6</sup> is

a) hydrogen

b) straight or branched alkyl (C1-C6) which may be substituted by

i) phenyl

ii) phenyl substituted with straight or branched alkyl (C1-C6), straight or branched alkoxy (C1-C6)

iii) pyridyl

c) phenyl, naphthyl, furyl, thiofuryl, cycloalkyl (C5-C8) bicycloalkyl (C6-C10), tricycloalkyl (C7-C12), tetracycloalkyl (C10-C16), pentacycloalkyl (C11-C20) or benzoyl. These groups may be substituted by

i) amine,

ii) amino substituted by a straight or branched alkoxycarbonyl (C1-C6) that may be substituted by phenyl or an alkene (C2-C6).

iii) amino substituted by alkanoyl (C1-C6), or benzoyl

iv) sulfonamide (-SO<sub>2</sub>NH<sub>2</sub>)

v) hydroxy, or a straight or branched alkoxy (C1-C6), that may be substituted by phenyl

Included within the scope of the present invention are pharmaceutically acceptable salts of the above mentioned compounds. Pharmaceutically acceptable salts can be derived from mineral acids, carboxylic acids or sulfuric acids preferred from hydrochloric acid, hydrobromic acid, sulfuric acid, methane sulfuric acid, ethane sulfonic acid, toluene sulfonic acid, benzene sulfonic acid, naphthalene disulfonic acid, acetic

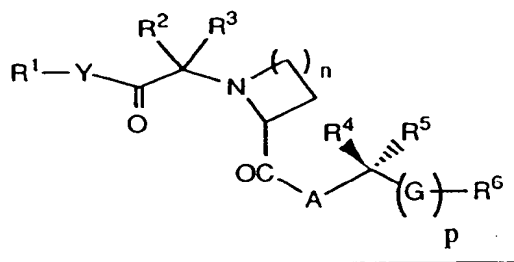


acid, propionic acid, lactic acid, tartaric acid, citric acid, fumaric acid, maleic acid or benzoic acid. Most preferred are the hydrochlorides.

In the case of the present compounds being carboxylic acids or containing acidic functional groups, the invention includes metal salts and ammonium salts. Preferred are sodium, potassium or ammonium salts.

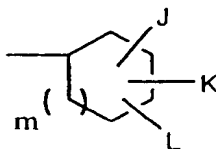
5 The compounds of this invention exist as stereoisomeric forms, which either behave like image and mirror image (enantiomers) or not (diastereomers). Included within the scope of the invention are the enantiomers, the racemic form as well as diastereomeric mixtures. Enantiomers as well as diastereomers can be separated by methods known to those skilled in the art (compare E.L. Eliel, Stereochemistry of Carbon Compounds, McGraw Hill, 1962).

10 Preferred compounds comprise at least one of the following structures:



where R<sup>1</sup> is

- 25 a) hydrogen,
- b) linear or branched alkyl (C1-C6) which may be substituted
  - i) once by hydroxy,
  - ii) once by phenyl which may be substituted by straight or branched alkyl (C1-C4), or straight or branched alkoxy (C1-C6),
  - 30 iii) once by cycloalkyl (C3-C8) which may be substituted by straight or branched alkyl (C1-C6), or straight or branched alkoxy (C1-C4),
  - iv) once by bicycloalkyl (C6-C10) which may be substituted by straight or branched alkyl (C1-C8), or straight or branched alkoxy (C1-C4),
  - v) once by tricycloalkyl (C7-C12) which may be substituted by straight or branched alkyl (C1-C6), or
  - 35 straight or branched alkoxy (C1-C4),
  - vi) once by tetracycloalkyl (C10-C12), which may be substituted by straight or branched alkyl (C1-C8), or straight or branched alkoxy (C1-C4),
  - vii) up to two times by phenyl and cycloalkyl (C5-C7), or
  - viii) up to two times by phenyl and morpholinyl.
- 40 c) alkene (C3-C8), which may be substituted by phenyl, straight or branched alkyl (C1-C4), or straight or branched alkoxy (C1-C4),
- d) diene (C4-C7) substituted by straight or branched alkyl (C1-C6), or straight or branched alkoxy (C1-C4),
- e) triene (C10-C16) substituted up to three times by straight or branched alkyl (C1-C6), or straight or
- 45 branched alkoxy (C1-C4),
- f) cycloalkyl (C5-C10), or the cycloalkyl fragment



55 where

m is an integer of 0, 1, or 2;

J, K, and L are independently or simultaneously

i) hydrogen

ii) straight or branched alkyl (C1-C5), which may be substituted by phenyl, or straight or branched alkoxy (C1-C4).

iii) phenyl, or

iv) phenyl substituted by straight or branched alkyl (C1-C4), or chlorine, or straight or branched alkoxy (C1-C4).

g) bicycloalkyl (C7-10) which may be substituted up to 3 times with straight or branched alkyl (C1-C6), or straight or branched alkoxy (C1-C4).

h) tricycloalkyl (C7-14) which may be substituted up to 3 times with straight or branched alkyl (C1-C6), or straight or branched alkoxy (C1-C4).

i) tetracycloalkyl (C10-C15) which may be substituted up to 3 times by straight or branched alkyl (C1-C6), or straight or branched alkoxy (C1-C4).

j) naphthyl, or the heteroaryl derivatives benzothieryl, benzofuryl, benzopyranyl, furyl, pyridyl, pyranyl, or 1,3-oxazolyl. These derivatives may be substituted up to two times by

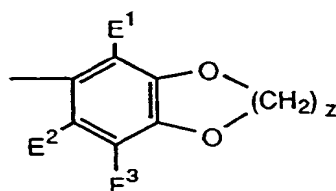
i) straight or branched alkyl (C1-C6)

ii) halogen.

iii) or both

k) 1,2,3,4-tetrahydronaphthyl.

l) the piperonyl fragment

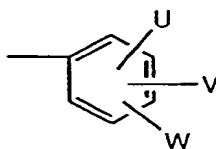


where

z is an integer of 1, or 2;

and E<sup>1</sup>, E<sup>2</sup>, and E<sup>3</sup> can be independently or simultaneously hydrogen, straight or branched alkyl (C1-C4), straight or branched alkoxy (C1-C4), or chlorine.

m) the aryl derivative



where

U, V, and W can be independently or simultaneously

i) hydrogen

ii) straight or branched alkyl (C1-C4), which may be substituted by phenyl

iii) straight or branched alkoxy (C1-C6) which may be substituted by phenyl, straight or branched alkoxy (C1-C4), or phenoxy.

iv) hydroxy,

v) phenyl,

vi) halogen

vii) nitro,

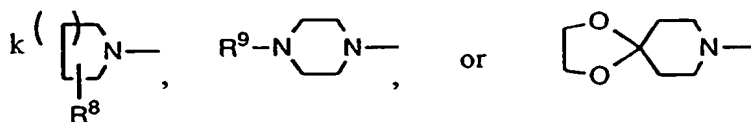
viii) benzoyl,

ix) phenoxy.

Y is a covalent bond, oxygen, NR<sup>7</sup>, where R<sup>7</sup> is hydrogen.

in addition to that described above,

R<sup>1</sup>-Y- may also be



where

k is an integer of 1, or 2;

R<sup>8</sup> is

- a) hydrogen.
- b) carboalkoxy with a straight or branched alkoxy (C1-C4)
- c) straight or branched alkyl (C1-C4) which may be substituted by phenyl, or straight or branched alkoxy (C1-C4)
- d) phenyl, or phenyl substituted by halogen

R<sup>9</sup> is phenyl which may be substituted by alkyl (C1-C4).

R<sup>2</sup> and R<sup>3</sup> are defined as follows: one of R<sup>2</sup> and R<sup>3</sup> are hydrogen, and the other is hydrogen or straight or branched alkyl (C1-C6).

n is an integer of 2 or 3.

A is oxygen or NR<sup>10</sup>, where R<sup>10</sup> is hydrogen or straight or branched alkyl (C1-C4).

R<sup>4</sup> is

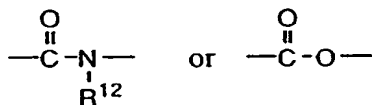
- a) hydrogen.
  - b) straight or branched alkyl (C1-C6) which may be substituted by
    - i) phenyl, or phenyl substituted by hydroxy or methoxy
    - ii) cycloalkyl (C5-C6)
    - iii) alkylthio (C1-C6)
    - iv) carboxamido
    - v) straight or branched alkoxy (C1-C6) which may be substituted by phenyl
  - c) phenyl
  - d) cycloalkyl (C3-C7), which may be substituted by straight or branched alkyl (C1-C6)
- R<sup>5</sup> is hydrogen or straight or branched alkyl (C1-C4). In addition, R<sup>4</sup> and R<sup>5</sup>, taken together can be -(CH<sub>2</sub>)<sub>r</sub>-

where r is an integer of 4 or 5.

G is one of the fragments

-HC=CH-, -CH<sub>2</sub>•CH<sub>2</sub>-, or -CH<sub>2</sub>-

or one of the fragments



where it is understood that the carbonyl group is attached to the carbon bearing R<sup>4</sup> and R<sup>5</sup> and that NR<sup>12</sup> and oxygen are connected to R<sup>6</sup>, respectively, R<sup>12</sup> is hydrogen or methyl.

p is an integer of 0 or 1.

R<sup>6</sup> is

- a) hydrogen
- b) straight or branched alkyl (C1-C4) which may be substituted by
  - i) phenyl
  - ii) phenyl substituted with straight or branched alkyl (C1-C4), straight or branched alkoxy (C1-C4)
  - iii) 2- or 4-pyridyl
- c) phenyl or naphthyl, which may be substituted by
  - i) amine,
  - ii) amino substituted by a straight or branched alkoxy carbonyl (C1-C6) that may be substituted by phenyl or an alkene (C2-C6).
  - iii) amino substituted by alkanoyl (C1-C6), or benzoyl
  - iv) sulfonamide (-SO<sub>2</sub>NH<sub>2</sub>)

v) straight or branched alkoxy (C1-C6), that may be substituted by phenyl

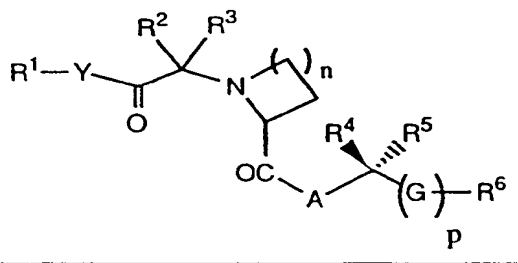
d) benzoyl

e) furyl, or thiofuryl

f) cycloalkyl (C5-C8), bicycloalkyl (C6-C10), tricycloalkyl (C7-C12), or tetracycloalkyl (C10-C14)

Included within the scope of the present invention are pharmaceutically acceptable salts of the above mentioned compounds. Pharmaceutically acceptable salts can be derived from mineral acids, carboxylic acids or sulfuric acids preferred from hydrochloric acid, hydrobromic acid, sulfuric acid, methane sulfuric acid, ethane sulfonic acid, toluene sulfonic acid, benzene sulfonic acid, naphthalene disulfonic acid, acetic acid, propionic acid, lactic acid, tartaric acid, citric acid, fumaric acid, maleic acid or benzoic acid. Most preferred are the hydrochlorides

Most preferred are compounds comprising at least one of the following structures:



where

R<sup>1</sup> is

a) hydrogen,

b) linear or branched alkyl (C1-C6) which may be substituted by

i) hydroxy,

ii) phenyl, or phenyl substituted by straight or branched alkyl (C1-C4)

iii) cycloalkyl (C3-C8) which may be substituted by straight or branched alkyl (C1-C4)

iv) bicycloalkyl (C6-C9) which may be substituted by straight or branched alkyl (C1-C6)

v) tricycloalkyl (C7-C12) which may be substituted by straight or branched alkyl (C1-C4)

vi) tetracycloalkyl (C10-C12), which may be substituted by straight or branched alkyl (C1-C6)

vii) both phenyl and cycloalkyl (C5-C6), or

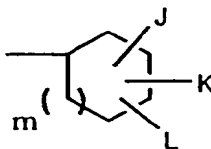
viii) both phenyl and morpholinyl.

c) alkene (C3-C6), which may be substituted by phenyl.

d) diene (C5-C6) substituted by straight or branched alkyl (C1-C4)

e) triene (C13-C16) substituted up to three times by straight or branched alkyl (C1-C4).

f) cycloalkyl (C5-C6), or the cycloalkyl fragment



where

m is an integer of 0, 1, or 2;

J, K, and L are independently or simultaneously

i) hydrogen

ii) straight or branched alkyl (C1-C5),

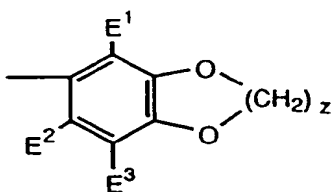
iii) phenyl, or

iv) phenyl substituted by straight or branched alkyl (C1-C4), or chlorine, or straight or branched alkoxy (C1-C4).

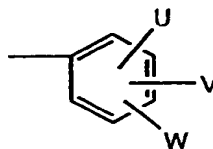
g) bicycloalkyl (C7-8) which may be substituted up to 3 times with straight or branched alkyl (C1-C4).

h) tricycloalkyl (C7-12) which may be substituted up to 2 times with straight or branched alkyl (C1-C6).

- i) tetracycloalkyl (C10-C12) which may be substituted up to 3 times by straight or branched alkyl (C1-C4).
- j) 2-benzothieryl substituted independently or simultaneously at least twice by either
  - i) straight or branched alkyl (C1-C3)
  - ii) chlorine
  - iii) or both
- k) 2-furyl.
- l) 2-pyridyl.
- m) 2-naphthyl.
- n) 1,2,3,4-tetrahydronaphthyl.
- o) 2-benzopyranyl.
- p) 2-benzofuryl.
- q) the piperonyl fragment

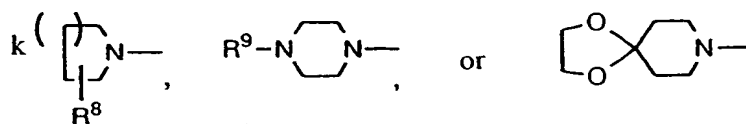


where  
 z is an integer of 1, or 2;  
 and E<sup>1</sup>, E<sup>2</sup>, and E<sup>3</sup> are hydrogen  
 r) the aryl derivative



where  
 U, V, and W can be independently or simultaneously  
 i) hydrogen  
 ii) straight or branched alkyl (C1-C4),  
 iii) straight or branched alkoxy (C1-C4),  
 iv) alkoxy (C2) substituted by alkoxy (C2), or phenoxy.  
 v) hydroxy,  
 vi) phenyl,  
 vii) fluorine,  
 viii) chlorine,  
 ix) bromine,  
 x) nitro,  
 xi) benzyloxy,  
 xii) benzoyl,  
 xiii) phenoxy.

Y is a covalent bond, oxygen, NR<sup>7</sup>, where R<sup>7</sup> is hydrogen.  
 in addition to that described above,  
 R<sup>1</sup>-Y- may also be



where

k is an integer of 1, or 2;

R<sup>8</sup> is

- a) hydrogen.
- b) carboalkoxy with alkoxy (C1-C2)
- c) straight or branched alkyl (C1-C4) which may be substituted by phenyl.
- d) phenyl

R<sup>9</sup> is phenyl

- 15 R<sup>2</sup> and R<sup>3</sup> are defined as follows: one of R<sup>2</sup> and R<sup>3</sup> is hydrogen, and the other is hydrogen or straight or branched alkyl (C1-C4).

n is an integer of 2 or 3.

A is oxygen or NR<sup>10</sup>, where R<sup>10</sup> is hydrogen or methyl.

R<sup>4</sup> is

- 20 a) hydrogen.
- b) straight or branched alkyl (C1-C4) which may be substituted by
  - i) phenyl
  - ii) cycloalkyl (C5-C6)
  - iii) alkylthio (C1-C4)
  - 25 iv) carboxamido
  - v) benzyloxy
- c) phenyl

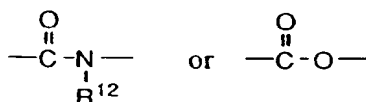
R<sup>5</sup> is hydrogen or straight or branched alkyl (C1-C4). In addition, R<sup>4</sup> and R<sup>5</sup>, taken together can be -(CH<sub>2</sub>)<sub>r</sub>--

- 30 where r is an integer of 5.

G is one of the fragments

-HC=CH-, -CH<sub>2</sub>-CH<sub>2</sub>-, or -CH<sub>2</sub>-

or one of the fragments



where it is understood that the carbonyl group is attached to the carbon bearing R<sup>4</sup> and R<sup>5</sup> and that NR<sup>12</sup> and oxygen are connected to R<sup>6</sup>, respectively, R<sup>12</sup> is hydrogen or methyl.

p is an integer of 0 or 1.

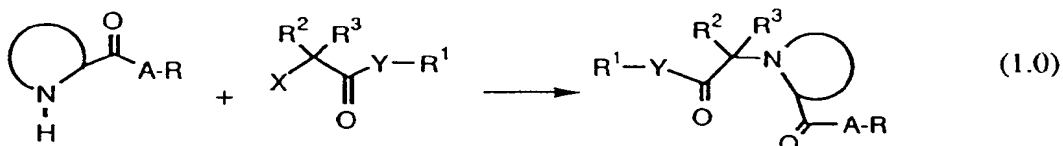
R<sup>6</sup> is

- 45 a) hydrogen
- b) straight or branched alkyl (C1-C4) which may be substituted by
  - i) phenyl
  - ii) phenyl substituted with alkoxy (C1-C2)
  - iii) 2- or 4-pyridyl
  - 50 c) phenyl which may be substituted by
    - i) amino,
    - ii) amino substituted by allyloxycarbonyl
    - iii) amino substituted by acetyl
    - iv) amino substituted by benzoyl
    - 55 v) amino substituted by benzyloxycarbonyl
    - iii) sulfonamide (-SO<sub>2</sub>NH<sub>2</sub>)
    - iv) straight or branched alkoxy (C1-C4)
    - d) benzoyl

- e) furyl
- f) naphthyl
- g) cycloalkyl (C5-C8)
- h) tetracycloalkyl (C10-C12)

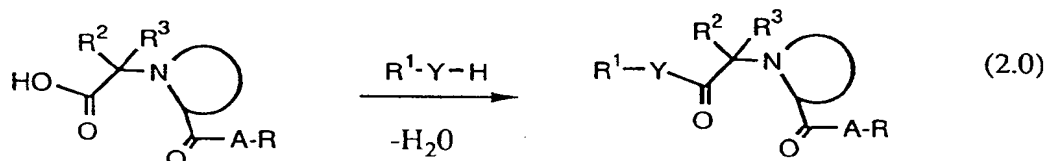
Included within the scope of the present invention are pharmaceutically acceptable salts of the above mentioned compounds. Most preferred are the hydrochlorides.

#### Preferred Method of Synthesis

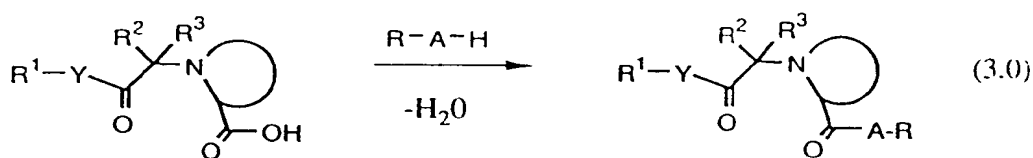


A convenient route to prepare the present compounds was to alkylate selected cyclic imino acids with  $\alpha$ -substituted methyl carbonyl compounds (Eq. 1.0). The  $\alpha$ -substitution could be in the form of halides such as chloride, bromide and iodide, but can be extended to other groups that are amenable for displacement. Solvents useful for effecting this transform include ethyl ether, tetrahydrofuran, alcohol solvents, or nitrile solvents such as acetonitrile. In certain cases, it may be advantageous not to use solvents. There are a number of possible conditions and variations that could be used for this type of synthesis route, such possibilities being well known to those skilled in the art. (For example, see Miyazawa, 1980, T. Bull. Chem. Soc. Japan 53:2555).

When properly substituted, the products from the reaction depicted in Eq. 1.0 can themselves serve as intermediates for the synthesis of other analogs. Hence 2-alkoxy derivatives of these 2-oxoethyl analogs can be converted to the corresponding acid derivatives using methods known in the art (T. W. Greene et al, Protective Groups in Organic Synthesis, 2nd Edition; John Wiley & Sons, 1991). The acid functionality that results may be converted to an activated acyl derivative and coupled to an appropriate Y-R1 derivative using methods described earlier (Bodanszky The Practice of Peptide Synthesis: Springer-Verlag, Vol 21, 1984).



In another embodiment, substituted 2-oxoethyl derivatives may be converted to active embodiments of this invention by coupling to an appropriate A-R derivative using methods described in the literature (Bodanszky The Practice of Peptide Synthesis: Springer-Verlag, Vol 21, 1984).



The alpha-halo ketones and esters used are either commercially available or can be prepared from steps available in current literature. For example, the alpha-halo esters can be prepared from the corresponding alcohols by treatment with alpha-halo acetylhalides such as alpha-chloro acetylchloride, and the alpha-halo ketones can be prepared from the corresponding carboxylic acids. Thus, the carboxy groups are transformed into either an acid chloride or an anhydride and treated with diazomethane to provide the corresponding alpha-diazo ketone. The diazo ketones are converted to alpha-halo ketones upon treatment with hydrogen halides such as HCl.

The presently claimed compounds were found to be effective at low micromolar doses in both in vitro PPIase enzyme inhibition assays and in vivo assays for inhibition of mitogen-induced human T-cell proliferation. Moreover, the results from the graft vs. host assay (described in detail further below) indicate that the present class of compounds exhibit desirable biological properties (prophylactic prevention of lymph node swelling), with no obvious toxicity at 100 mg/kg concentrations.

The present invention encompasses pharmaceutical formulations which, in addition to non-toxic, inert pharmaceutically suitable excipients, contain the compounds of the invention.

The present invention also includes pharmaceutical formulations in dosage units. This means that the formulations are present in the form of individual part, for example, tablets, dragees, capsules, caplets, pills, suppositories and ampules, the active compound content of which corresponds to a fraction or a multiple of an individual dose. The dosage units can contain, for example, 1, 2, 3 or 4 individual doses; or 1/2, 1/3 or 1/4 of an individual dose. An individual dose preferably contains the amount of active compound which is given in one administration and which usually corresponds to a whole, one half, one third or one quarter of a daily dose.

By non-toxic inert pharmaceutically suitable excipients there are to be understood solid, semi-solid or liquid diluents, fillers and formulation auxiliaries of all types.

Preferred pharmaceutical formulations which may be mentioned are tablets, dragees, capsules, caplets, pills, granules, suppositories, solutions, suspensions and emulsions, paste, ointments, glues, creams, lotions, dusting powders and sprays. Tablets, dragees, capsules, caplets, pills and granules can contain the active compounds in addition to the customary excipients, such as (a) fillers and extenders, for example, starches, lactose, sucrose, glucose, mannitol and silicic acid, (b) binders, for example, carboxymethylcellulose, alginates, gelatin and polyvinylpyrrolidone, (c) humectants, for example, glycerol, (d) disintegrating agents, for example, agar-agar, calcium carbonate and sodium carbonate, (e) solution retarders, for example, paraffin and (f) absorption accelerators, for example, quaternary ammonium compounds, (g) wetting agents, for example, cetyl alcohol and glycerol monostearate, (h) absorbents, for example, kaolin and bentonite and (i) lubricants, for example, talc, calcium stearate, magnesium stearate and solid polyethylene glycols, or mixtures of the substances listed under (a) to (i) directly hereinabove.

The tablets, dragees, capsules, caplets, pills and granules can be provided with the customary coatings and shells, optionally containing opacifying agents and can also be of such composition that they release the active compounds only or preferentially in a certain part of the intestinal tract, optionally in a delayed manner. Examples of embedding compositions which can be used are polymeric substances and waxes.

The active compounds can also be present in microencapsulated form, if appropriate with one or more of the abovementioned excipients.

Suppositories can contain, in addition to the active compounds, the customary water-soluble or water-insoluble excipients, for example, polyethylene glycols, fats, for example, cacao fat and higher esters (for example, C<sub>14</sub> -alcohol with C<sub>16</sub> -fatty acid), or mixtures of these substances.

Ointments, pastes, creams and gels can contain, in addition to the active compounds, the customary excipients, for example, animal and vegetable fats, waxes, paraffins, starch tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures of these substances.

Dusting powders and sprays can contain, in addition to the active compounds, the customary excipients, for example, lactose, talc, silicic acid, aluminum hydroxide, calcium silicate and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants, for example, chlorofluorohydrocarbons.

Solutions and emulsions can contain, in addition to the active compounds, customary excipients, such as solvents, solubilizing agents and emulsifiers, for example, water, ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils, in particular, cottonseed oil, groundnut oil, corn germ oil, olive oil, castor oil and sesame oil, glycerol, glycerol formal, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, or mixtures of these substances.

For parenteral administration, the solutions and emulsions can also be in a sterile form which is isotonic with blood.

Suspensions can contain, in addition to the active compounds, customary excipients, such as liquid diluents, for example, water, ethyl alcohol or propylene glycol and suspending agents, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum methyldioxide, bentonite, agar-agar, and tragacanth, or mixtures of these substances.

The abovementioned pharmaceutical formulations can also contain other pharmaceutical active compounds in addition to the claimed compounds of the present invention.



The aforementioned pharmaceutical formulations are prepared in the customary manner by known methods, for example, by mixing the active compound or compounds with the excipient or excipients.

The formulations mentioned can be used either with humans and animals, orally, rectally, buccally, parenterally (intra-venously, intramuscularly or subcutaneously), intracisternally, intravaginally, intraperitoneally or locally (dusting powder, ointment or drops) and for the therapy of infection in hollow spaces or body cavities. Suitable formulations are injection solutions, solutions and suspensions for oral therapy, gels, pour-on formulations, emulsions, ointments or drops. Ophthalmological and dermatological formulations, silver salts and other salts, ear drops, eye ointments, powders or solutions can be used for local therapy.

It is furthermore possible to use gels, powders, dusting powders, tablets, sustained release tablets, premixes, concentrates, granules, pellets, capsules, caplets, aerosols, sprays and inhalates on humans and animals. The compounds according to the invention can furthermore be incorporated into other carrier materials, such as, for example, plastics (e.g., chains of plastic for local therapy), collagen or bone cement.

## DETAILED DESCRIPTION

The following describes a preferred way to prepare the compounds of the present invention.

### REAGENTS AND INSTRUMENTS:

Anhydrous tetrahydrofuran (THF), ethyl ether (Et<sub>2</sub>O), and acetonitrile were distilled from calcium hydride prior to use. Unless otherwise stated, all reagents discussed in the following examples were commercially available from Aldrich Chemical Co, Milwaukee, WI, or Janssen Chimica through the U.S. vendor Spectrum Chemicals Mfg. Corp., New Brunswick, NJ. The general procedure for converting methyl-ketones to  $\alpha$ -bromoketones (unless otherwise specified) was according to steps described in Jaques et al., 1988, Org. Synth. Coll. 6:175-178.

All reactions were carried out in oven-dried glassware (140 °C) which were cooled under argon prior to use. Crude products were purified by flash column chromatography using 230-400 mesh silica gel (35-70  $\mu$ m) or medium/high pressure liquid chromatography using Shimadzu LC-8A Preparative liquid chromatography system equipped with columns packed with either 20  $\mu$ m or 10  $\mu$ m silica. Thin layer chromatography (TLC) was performed on aluminum-backed silica gel plates, and visualization was accomplished with a UV light or an iodine vapor chamber.

Proton (<sup>1</sup>H) nuclear magnetic resonance (NMR) spectra were obtained on GE-OMEGA-300 spectrometers at 300 MHz. Carbon (<sup>13</sup>C) NMR were obtained on these same spectrometers at 75 MHz. Mass spectral data were obtained on a Kratos-MS 80RFA spectrometer using electron impact ionization (EI), chemical ionization (CI), or fast atom bombardment (FAB). Mass Spectral (MS) data were obtained on a Kratos CONCEPT I-H spectrometer, using liquid-cesium secondary ion (LSI) technique, a more modern version of fast atom bombardment (FAB).

Melting points were obtained on a Thomas Hoover capillary melting point apparatus in open-ended capillaries and are not corrected.

### General Process for Preparing $\alpha$ -halo Ketones from Carboxylic Acids.

**2-Chloro-4'-(n-Pentyl) Acetophenone.** A solution of 4-pentyl-benzoic acid (1.178 g, 6.13 mmol) and oxalyl chloride (630  $\mu$ L, 855 mg, 6.74 mmol, 1.1 eq) in dichloromethane (15 mL) was stirred at 22 °C for 10 min, then treated with one drop of N,N-dimethylformamide at 22 °C {Caution, gas evolution may become brisk}. After gas evolution was no longer observed, the flask was fitted with a condenser and warmed to reflux for 30 min. The solution was cooled to -5 °C, and cannulated into a cold (-5 °C), ethereal solution of diazomethane (40 mL). After the solution was stirred at -5 °C for 30 min, the flask was removed from the cold bath and the yellow solution was allowed to stir at 22 °C for 2 hrs (preferably in the dark). The solution was concentrated in vacuo, and purified by flash chromatography (5% ethyl acetate in hexane) to provide 33 mg (1.3%) of 2-chloro-4'-(n-pentyl) acetophenone and 586 mg (44%) of 4'-(n-pentyl) diazoacetophenone as a bright yellow oil.

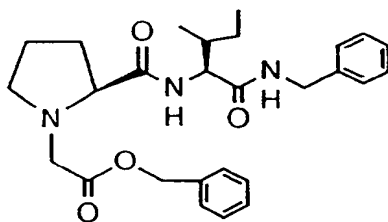
R<sub>f</sub> (10% ethyl acetate in hexane) = 0.37

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  7.65 (d, J = 8.1 Hz, 2 H), 7.20 (d, J = 7.9 Hz, 2 H), 5.93 (s, 1 H CHN<sub>2</sub>), 2.60 (m, 2 H), 1.58 (m, 2 H), 1.27 (m, 4 H), 0.86 (t, J = 6.9 Hz, 3 H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  186.78 (C=O), 149.05, 134.92, 129.31, 127.43, 54.47 (C=N<sub>2</sub>), 36.53, 32.06, 31.46, 23.14, 14.64.

The diazo compound was quickly taken up in ethyl acetate (120 mL), and the solution was cooled to -5 °C. To this was added a 1.0 M solution of HCl in ether (Aldrich, 6.0 mL). Gas evolution was observed, and the yellow solution became colorless. The flask was removed from the cold bath and the solution was allowed to stir at 22 °C for 2 hrs. The solution was poured into a separatory funnel, washed with satd aq NaHCO<sub>3</sub>, dried (MgSO<sub>4</sub>) and concentrated in vacuo to provide 0.595 g (98% based on starting diazo compd.) of 2-chloro-4'-(n-pentyl) acetophenone as a colorless oil.

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 7.82 (d, J = 6.5 Hz, 2 H), 7.23 (d, J = 6.5 Hz, 2 H), 4.64 (s, 2 H, CH<sub>2</sub>Cl), 2.60 (m, 2 H), 1.58 (m, 2 H), 1.28 (m, 4 H), 0.86 (t, J = 7.1 Hz, 3 H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 191.25 (C=O), 150.55, 132.55, 129.55, 129.27, 46.85 (CH<sub>2</sub>Cl), 36.63, 32.06, 31.34, 23.14, 14.65.

### Example 1



### L-Isoleucine, N-[1-(2-Benzoyloxy-2-Oxoethyl)-L-Prolyl] Benzylamide.

a) N-(tert-Butoxycarbonyl)-L-Isoleucine Benzylamide. Into a 500 mL round bottomed flask equipped with a magnetic stirrer was added N-(tert-butoxycarbonyl)-L-isoleucine (22.53 g, 97.39 mmol, 1.0 eq) and THF (300 mL). The solution was stirred until homogeneous, cooled to -5 °C, and treated with N-ethylmorpholine (14.23 mL, 12.88 g, 112.0 mmol, 1.15 eq). The solution was stirred at -5 °C for 20 min, and isobutyl chloroformate (13.24 mL, 13.90 g, 102 mmol, 1.05 eq) was added dropwise over 10 min. After stirring at -5 °C for 30 min, benzylamine (12.24 mL, 12.0 g, 112.0 mmol, 1.15 eq) was added dropwise over 10 min. After the addition was complete, the flask was removed from the cold bath and the solution was stirred at 22 °C for 2.5 hrs. The solution was concentrated to a residue, and partitioned between ethyl acetate (200 mL) and water (100 mL). The aqueous layer was extracted with ethyl ether (2 X 100 mL) and discarded. The organic extracts were combined, washed with 1N HCl (5 X 50 mL), 1 N NaOH (3 X 50 mL), satd. aq NaCl (50 mL), and dried (MgSO<sub>4</sub>). The solution was concentrated in vacuo to provide 29.93 g (96 %) of N-(tert-butoxycarbonyl)-L-isoleucine benzylamide as a white solid.

mp = 125-126 °C

R<sub>f</sub>(100% ethyl acetate) = 0.74

R<sub>f</sub> (50 % ethyl acetate in hexane) = 0.60

b) L-Isoleucine Benzylamide. Into a 500 mL round bottomed flask equipped with a magnetic stirrer was added N-(tert-butoxycarbonyl)-L-isoleucine benzylamide (29.93 g, 93.53 mmol, 1.0 eq) and dichloromethane (300 mL). The solution was stirred at 22 °C for 10 min until homogeneous, and trifluoroacetic acid (43.22 mL, 63.97 g, 0.57 mol, 6.0 eq) was added (Caution: gas evolution may be brisk!). After TLC analysis indicated that the reaction was complete, the solution was concentrated to an oil, and used directly in the next experiment.

c) N-Carbobenzyloxy-L-Proline-L-Isoleucine Benzylamide. Into a 1-L round bottomed flask equipped with a magnetic stirrer was added N-carbobenzyloxy-L-proline (25.618 g, 102 mmol, 1.0 eq), and dichloromethane (300 mL). The solution was cooled to 0 °C, and oxalyl chloride (10.15 mL, 15.02 g, 118.31 mmol, 1.15 eq) was added. After stirring at 0 °C for 5 min, five drops of N,N-dimethylformamide were added (Caution: gas evolution may be brisk!). The solution was stirred at 0 °C for 5 min, the flask was removed from the cold bath, and the solution was stirred at 22 °C for 9 hrs. The solution was concentrated in vacuo to remove all volatiles, dissolved in fresh dichloromethane (300 mL), and cooled to -5 °C. This solution was cannulated into a cooled (0 °C) solution containing L-isoleucine N-benzylamide and triethylamine (75.71 g, 748 mmol) dissolved in dichloromethane (100 mL). After the addition was complete, the flask was removed from the cold bath, and the solution was stirred at 22 °C for 3 hrs. The solution was poured into a separatory funnel and washed with water (3 X 75 mL), 1 N HCl (7 X 100 mL), 1 N NaOH (4 X 100 mL), satd. aq NaCl (100 mL), and dried (MgSO<sub>4</sub>). The solution was concentrated in vacuo to provide a crude residue. The residue was recrystallized (ethyl acetate/hexane) to provide 28.72

g (68%) of the product as a white solid. The mother liquor was concentrated to an oil and purified by flash chromatography (20 % ethyl acetate) to provide 7.88 g (18.6%) of additional product, or 36.60 g (86%) of the title compound as a white solid.

mp = 151-153 °C

$R_f$  (50 % ethyl acetate in hexane) = 0.18

Mass Spectrum (+ EI) m/e (rel intensity) 451 (20, M<sup>+</sup>), 395 (18), 345 (8), 317 (100) 232 (42), 204 (63).

d) L-Proline-L-Isoleucine Benzylamide. A solution of N-carbobenzyloxy-L-proline-L-isoleucine benzylamide (36.60 g, 80.95 mmol), 10% palladium on carbon (0.957 g), and methanol (700 mL) was degassed and purged repeatedly (15 times) with hydrogen, and stirred under an atmosphere of hydrogen at 22 °C. When TLC analysis indicated the reaction was complete, the solution was purged with argon, filtered through a plug of celite, and concentrated in vacuo to provide 23.71 g, (92%) of the title compound as a white solid.

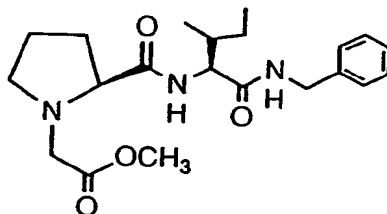
mp = 135-136 °C

$R_f$  (100% ethyl acetate) = 0.08

e) L-Isoleucine N-[1-(2-Benzyloxy-2-Oxoethyl)-L-Prolyl] Benzylamide. A solution of L-proline-L-isoleucine benzylamide (10.22 g, 32.20 mmol), and sodium carbonate (6.824 g, 64.39 mmol, 2.0 eq), in acetonitrile (150 mL) was warmed to reflux until homogeneous, cooled to 22 °C, and treated with benzyl 2-bromoacetate (14.75 g, 64.39 mmol, 2.0 eq). The flask was returned to the oil bath and warmed to reflux until TLC indicated the reaction was complete. The heterogeneous solution was filtered, concentrated to a residue, taken up in ethyl acetate (400 mL), and washed with satd aq NaHCO<sub>3</sub> (2 X 50 mL). The solution was dried (MgSO<sub>4</sub>), concentrated to a residue, and purified by flash chromatography (20% ethyl acetate in hexane) to provide 14.80 g (99%) of the title compound as a colorless oil. The <sup>1</sup>H NMR analysis of this compound was consistent with the structure.

$R_f$  (100% ethyl acetate) = 0.63

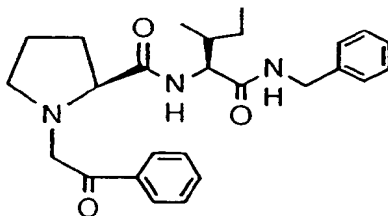
## Example 2



**L-Isoleucine, N-[1-(2-Methoxy-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of L-proline-L-isoleucine benzylamide (47 mg, 0.15 mmol), with sodium carbonate (31 mg, 0.29 mmol, 2.0 eq), and methyl alpha-bromoacetate (113 mg, 0.74 mmol, 5.0 eq) in acetonitrile (5 mL), provided 50 mg (87%) of the title compound as a white foam. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure.

$R_f$  (50% dichloromethane in ethyl acetate) = 0.50

## Example 3



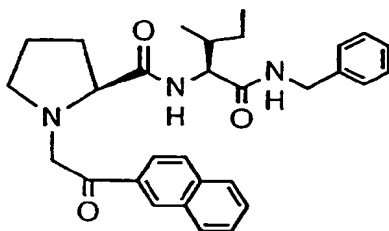
**L-Isoleucine, N-[1-(2-Phenyl-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of L-proline-L-isoleucine benzylamide (361 mg, 1.14 mmol), with cesium carbonate (0.74 g, 2.28 mmol), and 2-bromoacetophenone (1.134 g, 5.69 mmol, 5.0 eq) in acetonitrile (12 mL), provided 466 mg (94%) of the title compound as a pale yellow oil. The 300 MHz, <sup>1</sup>H NMR analysis of this

compound was consistent with the structure.

$R_f$  (50% ethyl acetate in hexane) = 0.14

$R_f$  (100% ethyl acetate) = 0.54

#### Example 4



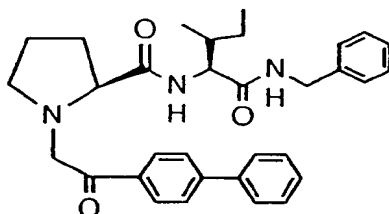
**L-Isoleucine, N-[1-(2-Naphth-2-yl-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of L-proline-L-isoleucine benzylamide (296 mg, 0.93 mmol), with cesium carbonate (0.61 g, 1.87 mmol), and 2-bromo-2'-acetonaphthone (697 mg, 2.80 mmol, 3.0 eq) in acetonitrile (12 mL), provided 364 mg (80%) of the title compound as a pale yellow oil. The 300 MHz,  $^1\text{H}$  NMR analysis of this

compound was consistent with the structure.

$R_f$  (50% ethyl acetate in hexane) = 0.25

$R_f$  (100% ethyl acetate) = 0.65

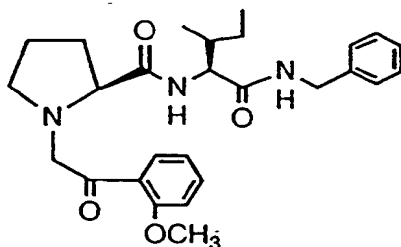
#### Example 5



**L-Isoleucine, N-[1-(2-(Biphenyl-4-yl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of L-proline-L-isoleucine benzylamide (300 mg, 0.95 mmol), with sodium carbonate (200 mg, 1.89 mmol), and 2-bromo-4'-phenylacetophenone (520 mg, 1.89 mmol, 2.0 eq) in acetonitrile (10 mL), provided 484 mg (87%) of the title compound as a colorless oil. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

$R_f$  (100% ethyl acetate) = 0.62

#### Example 6

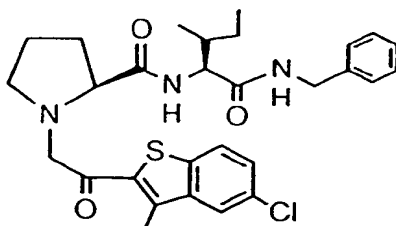


**L-Isoleucine, N-[1-(2-(2-Methoxyphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of L-proline-L-isoleucine benzylamide (317 mg, 0.99 mmol), with sodium carbonate (211 mg, 1.99 mmol), and 2-bromo-2'-methoxyacetophenone (457 mg, 1.99 mmol, 2.0 eq) in acetonitrile (10 mL), provided 410 mg (88%) of the title compound as a pale yellow oil. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

$R_f$  (100% ethyl acetate) = 0.46

Mass Spectrum (+ EI) m/e (rel intensity) 346 (100), 328 (38).

### Example 7



### 15 L-Isoleucine, N-[1-(2-(5-Chloro-3-Methyl-benzo[B]thiophene-2-yl)-2-Oxoethyl)-L-Prolyl] Benzylamide

Using the procedure described in example 1e, treatment of L-proline-L-isoleucine benzylamide (200 mg, 0.63 mmol), with sodium carbonate (100 mg, 0.94 mmol), and 2-chloroacetyl-5-chloro-3-methylbenzo[B]-thiophene (Ryan Scientific; Columbia, SC: 244 mg, 0.941 mmol, 1.5 eq) in acetonitrile (10 mL), provided 170 mg (50%) of the title compound as a pale yellow oil that formed a waxy solid on standing. The 300

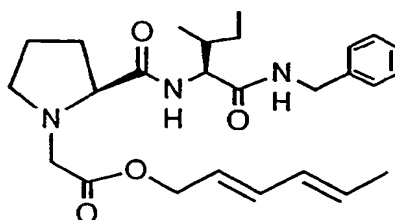
20 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

$R_f$  (50% ethyl acetate in hexane) = 0.17

$R_f$  (100% ethyl acetate) = 0.66

Mass Spectrum (+ EI) m/e (rel intensity) 540 (5,  $M^+$ ), 539 (10), 330 (100).

### 25 Example 8



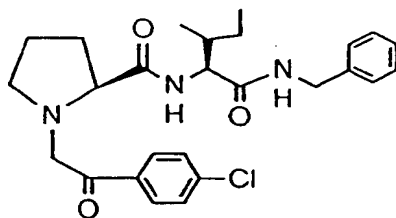
35 **L-Isoleucine, N-[1-(2-(trans,trans-Hexa-2,4-dienyl-1-oxy)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of L-proline-L-isoleucine benzylamide (201 mg, 0.63 mmol), with cesium carbonate (412 mg, 1.26 mmol), and 1-(2-chloroacetoxy)-2E,4E-hexadiene (prepared from 2E,4E-hexadien-1-ol and 2-chloro acetylchloride: 221 mg, 1.26 mmol, 2.0 eq) in acetonitrile (8 mL), provided 40 132 mg (46%) of the title compound as a colorless oil that formed a waxy solid on standing. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

$R_f$  (50% ethyl acetate in hexane) = 0.29

$R_f$  (70% ethyl acetate in hexane) = 0.43

Mass Spectrum (+ EI) m/e (rel intensity) 456 (5,  $M^+$ ), 374 (100), 330 (58).

### 45 Example 9

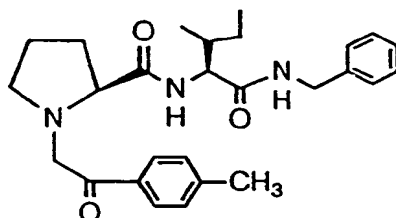


50 **L-Isoleucine, N-[1-(2-(4-Chlorophenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of L-proline-L-isoleucine benzylamide (182 mg, 0.57 mmol), with sodium

carbonate (60 mg, 0.57 mmol), and 2-bromo-4'-chloroacetophenone (147 mg, 0.63 mmol, 1.0 eq), in methanol (10 mL), provided 237 mg (88%) of the title compound as a pale yellow oil. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure.

R<sub>f</sub> (100% ethyl acetate) = 0.46

#### Example 10

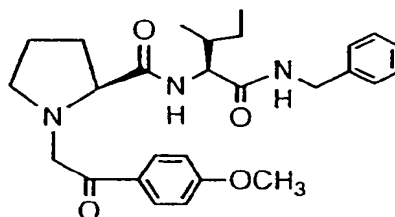


**L-Isoleucine, N-[1-(2-(4-Methylphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of L-proline-L-isoleucine benzylamide (191 mg, 0.60 mmol), with sodium carbonate (70 mg, 0.66 mmol), and 2-bromo-4'-methylacetophenone (141 mg, 0.66 mmol, 1.1 eq), in methanol (5 mL), provided 210 mg (78%) of the title compound as a pale yellow oil. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure.

R<sub>f</sub> (70% ethyl acetate in hexane) = 0.30

R<sub>f</sub> (100% ethyl acetate) = 0.52

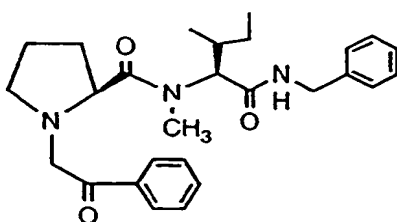
#### Example 11



**L-Isoleucine, N-[1-(2-(4-Methoxyphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of L-proline-L-isoleucine benzylamide (208 mg, 0.65 mmol), with sodium carbonate (104 mg, 0.98 mmol), and 2-bromo-4'-methoxyacetophenone (195 mg, 0.85 mmol, 1.3 eq) in MeOH (10 mL), provided 248 mg (81%) of the title compound as a pale yellow oil. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure.

R<sub>f</sub> (100% ethyl acetate) = 0.40

#### Example 12



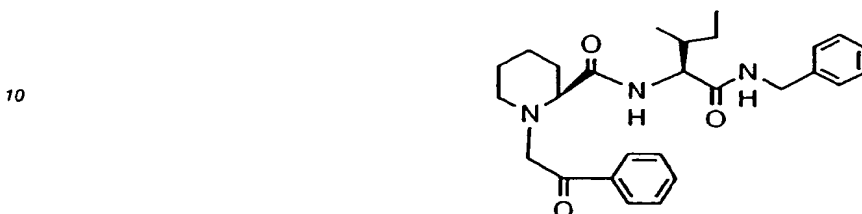
**L-Isoleucine, N-Methyl-N-[1-(2-Phenyl-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, 56 mg (0.17 mmol) of L-proline-L-(N-methyl)-isoleucine benzylamide (prepared from N-alpha-t-Boc-N-methyl-L-isoleucine (Schweizerhall, Piscataway, NJ.)) was treated with sodium carbonate (41 mg, 0.40 mmol), and 2-bromoacetophenone (52 mg, 0.26 mmol, 1.5 eq) in methanol (5 mL). A sample of the crude mixture was purified by preparative TLC to provide 2.2 mg of the title compound. The 300

MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

$R_f$  (20% ethyl acetate in dichloromethane) = 0.24

HRMS calcd for  $(\text{M} + \text{H})^+ [(\text{C}_{27}\text{H}_{36}\text{N}_3\text{O}_3 + \text{H})^+]$  ion 450.6057; found 450.2760

### 5 Example 13



15

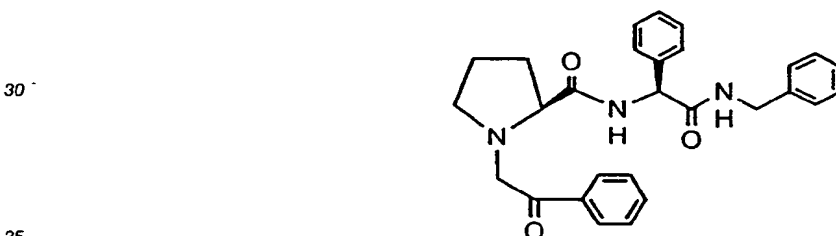
**L-Isoleucine, N-[1-(2-Phenyl-2-Oxoethyl)-L-Homoproline] Benzylamide.** Using the procedure described in example 1e, 55 mg (0.17 mmol) of L-homoproline-L-isoleucine benzylamide (prepared from L-homoproline (Bachem Bioscience, Philadelphia, PA.)), was treated with sodium carbonate (39 mg, 0.37 mmol), and 2-bromoacetophenone (59 mg, 0.29 mmol, 1.7 eq) in methanol (5 mL). A sample of the crude mixture

20 was purified by preparative TLC to provide 34 mg (45%) of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

$R_f$  (20% ethyl acetate in dichloromethane) = 0.31

HRMS calcd for  $(\text{M} + \text{H})^+ [(\text{C}_{27}\text{H}_{36}\text{N}_3\text{O}_3 + \text{H})^+]$  ion 450.6057; found 450.2760

### 25 Example 14



35

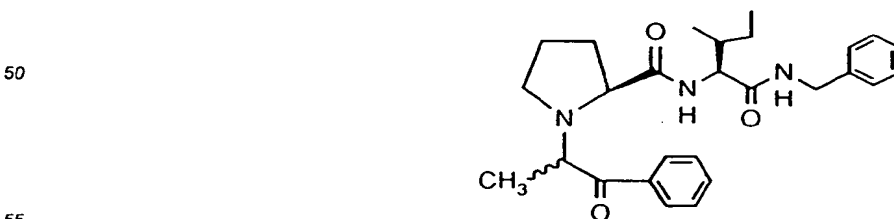
**L-Phenylglycine, N-[1-(2-Phenyl-2-Oxoethyl)-L-Proline] Benzylamide.** Using the procedure described in example 1e, 285 mg (0.84 mmol) of L-proline-L-phenylglycine benzylamide (prepared from L-phenylglycine (Bachem Bioscience, Philadelphia, PA.)), was treated with triethylamine (0.59 mL 4.23 mmol, 5 eq), and 2-bromoacetophenone (185 mg, 0.93 mmol, 1.1 eq) in THF (20 mL). A sample of the crude mixture was purified by preparative TLC to provide 50 mg of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

$R_f$  (50% ethyl acetate in hexane) = 0.09

HRMS calcd for  $(\text{M} + \text{H})^+ [(\text{C}_{28}\text{H}_{30}\text{N}_3\text{O}_3 + \text{H})^+]$  ion 456.5688; found 456.2289

45

### Example 15

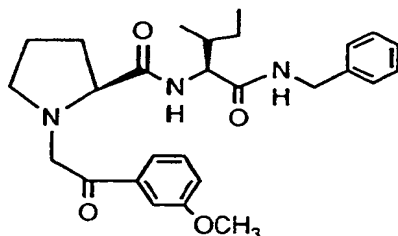


**L-Isoleucine, N-[1-(1-Methyl-2-Phenyl-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of L-proline-L-isoleucine benzylamide (196 mg, 0.62 mmol), with sodium

carbonate (85 mg, 0.80 mmol), and 2-bromopropiophenone (210 mg, 0.98 mmol, 1.6 eq) in MeOH (12 mL), provided 72 mg (26%) of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

$R_f$  (100% ethyl acetate) = 0.54

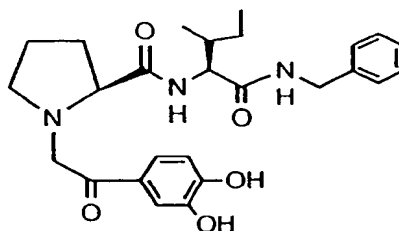
#### Example 16



**L-Isoleucine, N-[1-(2-(3-Methoxyphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of L-proline-L-isoleucine benzylamide (125 mg, 0.39 mmol), with triethylamine (275  $\mu\text{L}$ , 1.97 mmol, 5 eq), and 2-bromo-3'-methoxyacetophenone (107 mg, 0.47 mmol, 1.2 eq) in THF (20 mL), provided 149.2 mg (81%) of the title compound as a pale yellow oil. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

$R_f$  (50% ethyl acetate in dichloromethane) = 0.32

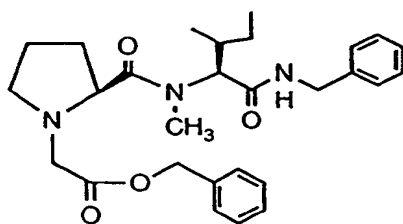
#### Example 17



**L-Isoleucine, N-[1-(2-(3,4-Dihydroxyphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of L-proline-L-isoleucine benzylamide (100 mg, 0.31 mmol), with triethylamine (220  $\mu\text{L}$ , 1.58 mmol, 5 eq), and 2-chloro-3'-4'-dihydroxyacetophenone (73 mg, 0.39 mmol, 1.2 eq) in THF (10 mL), provided 54 mg (81%) of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

$R_f$  (10% MeOH in dichloromethane) = 0.51

#### Example 18



**L-Isoleucine, N-Methyl-N-[1-(2-Benzoyloxy-2-Oxoethyl)-L-Prolyl] Benzylamid .** Using the procedure similar to that described in example 1e, treatment of L-proline-L-(N-methyl)-isoleucine benzylamide (43 mg, 0.13 mmol), with triethylamine (90  $\mu\text{L}$ , 0.65 mmol, 1.5 eq), and benzyl 2-bromoacetate (52 mg, 0.26 mmol, 2.0 eq) in THF (2.5 mL), provided 90 mg (64%) of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.



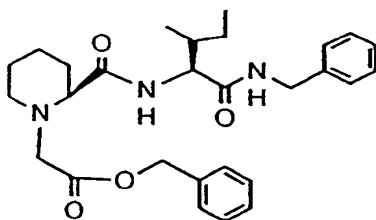
$R_f$  (50% ethyl acetate in dichloromethane) = 0.43

HRMS calcd for  $(M + H)^+$   $[(C_{28}H_{38}N_3O_4 + H)^+]$  ion 480.6328; found 480.2864.

### Example 19

5

10



15 **L-Isoleucine, N-[1-(Carbobenzyloxymethylene)-L-Homoproline] Benzylamide.** Using the procedure described in example 1e, L-homoproline-L-isoleucine benzylamide (43 mg, 0.13 mmol), was treated with triethylamine (91  $\mu$ L, 0.65 mmol, 1.5 eq), and benzyl 2-bromoacetate (41  $\mu$ L, 0.26 mmol, 2.0 eq) in THF (2.5 mL). Purification by HPLC provided 54.3 mg of the title compound. The 300 MHz,  $^1H$  NMR analysis of this compound was consistent with the structure.

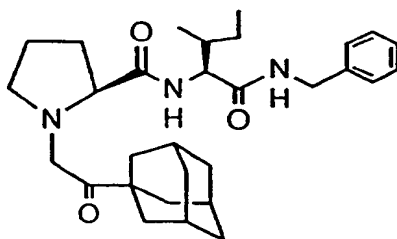
20  $R_f$  (50% ethyl acetate in dichloromethane) = 0.52

HRMS calcd for  $(M + H)^+$   $[(C_{28}H_{38}N_3O_4 + H)^+]$  ion 480.6328; found 480.2864.

### Example 20

25

30



35 **L-Isoleucine, N-[1-(2-Adamantan-1-yl-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of a solution of L-proline-L-isoleucine benzylamide (160.7 mg, 0.50 mmol), with triethylamine (3.53 mL, 2.53 mmol, 5.0 eq), and 1-adamantyl bromomethyl ketone (156 mg, 0.61 mmol, 1.2 eq) in THF (20 mL), provided 180 mg (99%) of the title compound as a white foam. The 300 MHz,  $^1H$  NMR analysis of this compound was consistent with the structure.

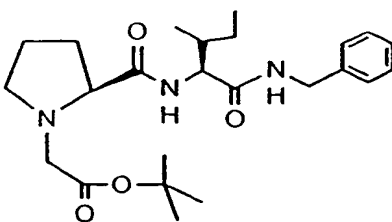
40  $R_f$  (50% dichloromethane in ethyl acetate) = 0.45

HRMS calcd for  $(M + H)^+$   $[(C_{30}H_{44}N_3O_3 + H)^+]$  ion 494.7030; found 494.3385.

### Example 21

45

50

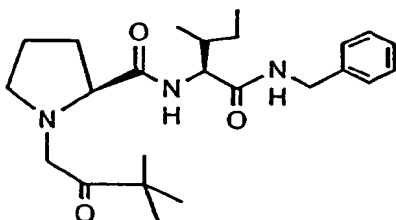


55 **L-Isoleucine, N-[1-(Carbo-tert-Butoxymethylene)-L-Prolin] Benzylamid.** Using the procedure described in example 1e, treatment of a solution of L-proline-L-isoleucine benzylamide (86 mg, 0.21 mmol), with triethylamine (147  $\mu$ L, 1.05 mmol, 5.0 eq), and alpha-bromo-tert-butylacetate (68  $\mu$ L, 0.42 mmol, 2 eq) in THF (5 mL), provided 70 mg (77%) of the title compound. The 300 MHz,  $^1H$  NMR analysis of this compound was consistent with the structure.

$R_f$  (50% dichloromethane in ethyl acetate) = 0.51

HRMS calcd for  $(M + H)^+$  [ $(C_{24}H_{38}N_3O_4 + H)^+$ ] ion 432.5884; found 432.2864

### Example 22

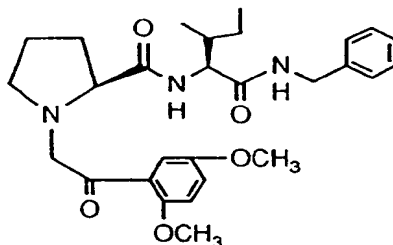


**L-Isoleucine, N-[1-(2-tert-Butyl-2-Oxoethyl)-L-Proline] Benzylamide.** Using the procedure described in example 1e, treatment of a solution of L-proline-L-isoleucine benzylamide (69 mg, 0.21 mmol), with triethylamine (151  $\mu$ L, 1.08 mmol, 5.0 eq), and 1-bromopinacolone (58  $\mu$ L, 0.43 mmol, 2 eq) in THF (5 mL), provided 35 mg (39%) of the title compound as a colorless oil. The 300 MHz,  $^1H$  NMR analysis of this compound was consistent with the structure.

$R_f$  (50% dichloromethane in ethyl acetate) = 0.42

HRMS calcd for  $(M + H)^+$  [ $(C_{24}H_{38}N_3O_4 + H)^+$ ] ion 416.5863; found 416.2915

### Example 23

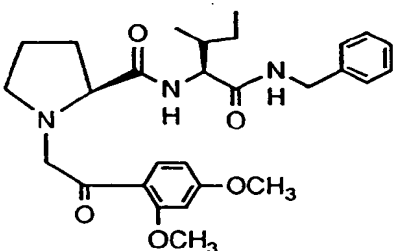


**L-Isoleucine, N-[1-(2-(2,5-Dimethoxyphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of a solution of L-proline-L-isoleucine benzylamide (51 mg, 0.16 mmol), with triethylamine (113  $\mu$ L, 0.81 mmol, 5.0 eq), and 2-bromo-2'-5'-dimethoxyacetophenone (50 mg, 0.19 mmol, 1.2 eq) in THF (5 mL), provided 60 mg (75%) of the title compound as a colorless oil. The 300 MHz,  $^1H$  NMR analysis of this compound was consistent with the structure.

$R_f$  (50% dichloromethane in ethyl acetate) = 0.36

HRMS calcd for  $(M + H)^+$  [ $(C_{28}H_{38}N_3O_5 + H)^+$ ] ion 496.6307; found 496.2813

### Example 24

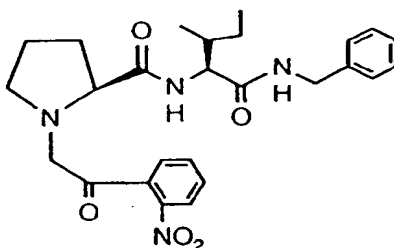


**L-Isoleucine, N-[1-(2-(2,4-Dimethoxyphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of a solution of L-proline-L-isoleucine benzylamide (51 mg, 0.16 mmol), with triethylamine (112  $\mu$ L, 0.80 mmol, 5.0 eq), and 2-bromo-2'-4'-dimethoxyacetophenone (50 mg, 0.19 mmol, 1.2 eq) in THF (5 mL), provided 55 mg (70%) of the title compound as a colorless oil. The 300 MHz,

<sup>1</sup>H NMR analysis of this compound was consistent with the structure.

R<sub>f</sub> (50% dichloromethane in ethyl acetate) = 0.34

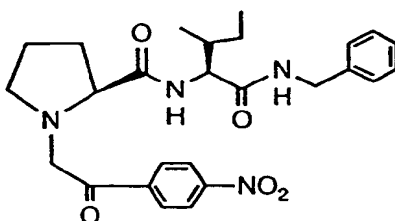
#### Example 25



**L-Isoleucine, N-[1-(2-(2-Nitrophenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of a solution of L-proline-L-isoleucine benzylamide (54 mg, 0.17 mmol), with triethylamine (118 uL, 0.84 mmol, 5.0 eq), and 2-bromo-2'-nitroacetophenone (50 mg, 0.20 mmol, 1.2 eq) in THF (5 mL), provided 46 mg (57%) of the title compound. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure.

R<sub>f</sub> (50% dichloromethane in ethyl acetate) = 0.29

#### Example 26

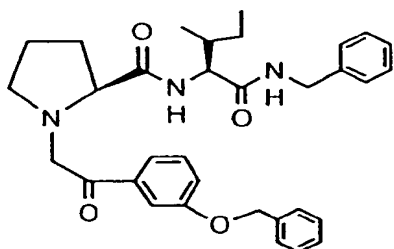


**L-Isoleucine, N-[1-(2-(4-Nitrophenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of a solution of L-proline-L-isoleucine benzylamide (50 mg, 0.16 mmol), with triethylamine (110 uL, 0.79 mmol, 5.0 eq), and 2-bromo-4'-nitroacetophenone (48 mg, 0.20 mmol, 1.2 eq) in THF (5 mL), provided crude material that was additionally purified by preparative TLC to provide 8.8 mg (12%) of the title compound. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure.

R<sub>f</sub> (50% dichloromethane in ethyl acetate) = 0.30

HRMS calcd for (M + H)<sup>+</sup> [(C<sub>26</sub>H<sub>33</sub>N<sub>4</sub>O<sub>5</sub> + H)<sup>+</sup>] ion 481.5754; found 481.2453

#### Example 27

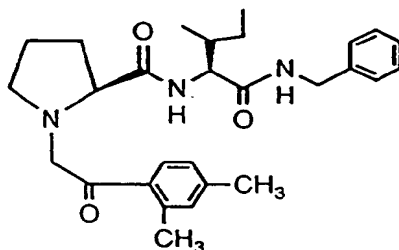


**L-Isoleucine, N-[1-(2-(3-Benzoyloxyphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of a solution of L-proline-L-isoleucine benzylamide (111 mg, 0.35 mmol), with triethylamine (245 uL, 1.75 mmol, 5.0 eq), and 2-bromo-3'-benzyloxyacetophenone (129 mg, 0.42 mmol, 1.2 eq) in THF (10 mL), provided 147 mg (76%) of the title compound. The 300 MHz, <sup>1</sup>H NMR analysis of

this compound was consistent with the structure.

$R_f$  (50% dichloromethane in ethyl acetate) = 0.38 HRMS calcd for  $(M+H)^+$   $[(C_{33}H_{40}N_3O_4 + H)^+]$  ion 542.7028; found 542.3021

#### Example 28

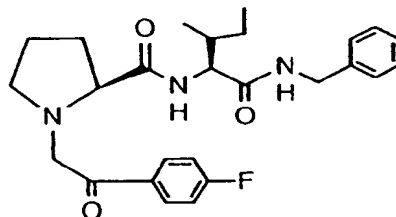


**L-Isoleucine, N-[1-(2-(2,4-Dimethylphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of a solution of L-proline-L-isoleucine benzylamide (60 mg, 0.19 mmol), with triethylamine (130  $\mu$ L, 0.94 mmol, 5.0 eq), and 2-bromo-2',4'-dimethylacetophenone (52 mg, 0.23 mmol, 1.2 eq) in THF (7 mL), provided a crude product. A portion of the product was purified by preparative TLC to provide 19 mg (21%) of the title compound. The 300 MHz,  $^1H$  NMR analysis of this compound was consistent with the structure.

$R_f$  (50% dichloromethane in ethyl acetate) = 0.38

HRMS calcd for  $(M+H)^+$   $[(C_{28}H_{38}N_3O_3 + H)^+]$  ion 464.6319; found 464.2915

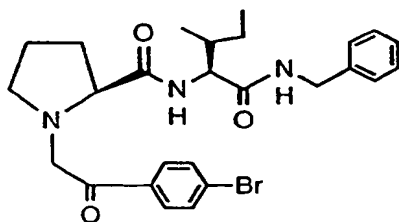
#### Example 29



**L-Isoleucine, N-[1-(2-(4-Fluorophenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of a solution of L-proline-L-isoleucine benzylamide (56 mg, 0.18 mmol), with triethylamine (123  $\mu$ L, 0.88 mmol, 5.0 eq), and 2-bromo-4'-fluoroacetophenone (37 mg, 0.21 mmol, 1.2 eq) in THF (7 mL), provided a crude product. A portion of the product was purified by preparative TLC to provide 28 mg (35%) of the title compound. The 300 MHz,  $^1H$  NMR analysis of this compound was consistent with the structure.

$R_f$  (50% dichloromethane in ethyl acetate) = 0.35

#### Example 30

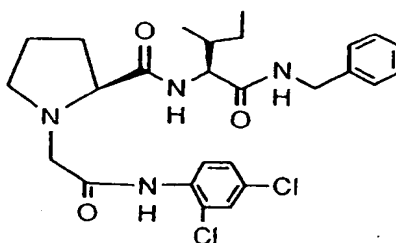


**L-Isoleucine, N-[1-(2-(4-Bromophenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of a solution of L-proline-L-isoleucine benzylamide (57 mg, 0.18 mmol),

with triethylamine (130  $\mu$ L, 0.93 mmol, 5.0 eq), and 2,4'-dibromoacetophenone (63 mg, 0.22 mmol, 1.2 eq) in THF (7 mL), provided a crude product. A portion of the product was purified by preparative TLC to provide 62 mg (67%) of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

5  $R_f$  (10% MeOH in dichloromethane) = 0.70

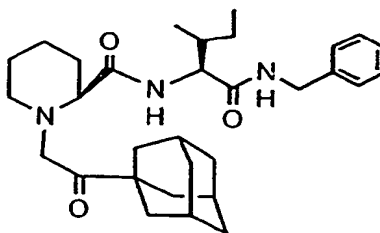
### Example 31



10 **L-Isoleucine, N-[1-(2,4-Dichlorophenylcarbamoylmethyl)-L-Proline] Benzylamide.** Using the procedure described in example 1e, treatment of a solution of L-proline-L-isoleucine benzylamide (58 mg, 0.18 mmol), with triethylamine (130  $\mu$ L, 0.93 mmol, 5.0 eq), and N-chloroacetyl-2,4-dichloroaniline (53 mg, 0.22 mmol, 1.2 eq) in THF (7 mL), provided a crude product. A portion of the product was purified by preparative TLC to provide 30 mg (32%) of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

15  $R_f$  (10% MeOH in dichloromethane) = 0.70

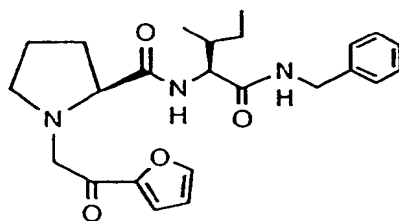
### Example 32



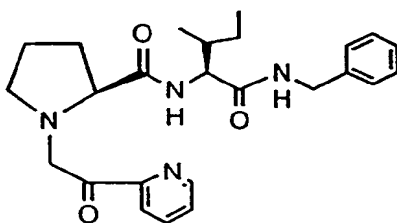
20 **L-Isoleucine, N-[1-(2-Adamantan-1-yl-2-Oxoethyl)-L-Homoproline] Benzylamide.** Using the procedure described in example 1e, L-homoproline-L-isoleucine benzylamide (75 mg, 0.22 mmol), was treated with triethylamine (0.15 mL, 1.12 mmol, 5 eq), 1-adamantyl bromomethyl ketone (92 mg, 0.36 mmol, 1.6 eq), and THF (10 mL), to provide 64 mg (56%) of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

$R_f$  (50% ethyl acetate in dichloromethane) = 0.56

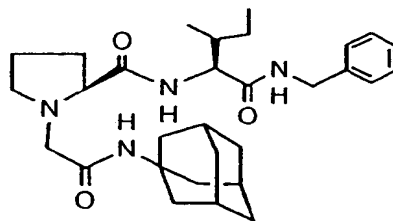
25 HRMS calcd for  $(M + H)^+$   $[(C_{31}H_{46}N_3O_3 + H)^+]$  ion 508.7290; found 508.3532

**Example 33**

**L-Isoleucine, N-[1-(2-Furan-2-yl-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, a solution of L-proline-L-isoleucine benzylamide (114 mg, 0.36 mmol), and triethylamine (0.10 mL, 0.78 mmol, 20 eq), in THF (10 mL), was treated with 255 mg (1.76 mmol, 5.0 eq) of 2-(alpha-chloroacetyl)furan (prepared from 2-furoic acid) to provide 130 mg, (85%) of the title compound. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure.  
 $R_f$  (100% ethyl acetate) = 0.25

**Example 34**

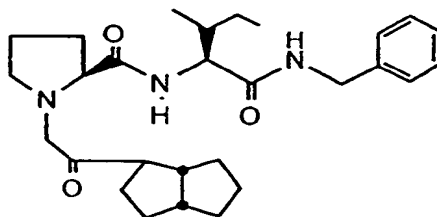
**L-Isoleucine, N-[1-(2-Pyrid-2-yl-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, a solution of L-proline-L-isoleucine benzylamide (148 mg, 0.47 mmol), and triethylamine (0.13 mL, 0.93 mmol, 2.0 eq) in THF (10 mL), was treated with 234 mg (1.50 mmol) of 2-(alpha-chloroacetyl)-pyridine (prepared from picolinic acid) to provide 20 mg, (10%) of the title compound. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure.  
 $R_f$  (50% ethyl acetate in hexane) 0.47

**Example 35**

**L-Isoleucine, N-[1-(Adamant-1-ylcarbamoylmethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, a solution of L-proline-L-isoleucine benzylamide (115 mg, 0.36 mmol), and triethylamine (253  $\mu$ L, 1.8 mmol, 5.0 eq) in THF (5 mL) was treated with 93 mg (0.43 mmol) of N-(alpha-chloroacetyl)1-aminoadamantane (prepared from 1-adamantanamine and 2-chloroacetyl chloride) to provide 135 mg, (73%) of the title compound. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure.

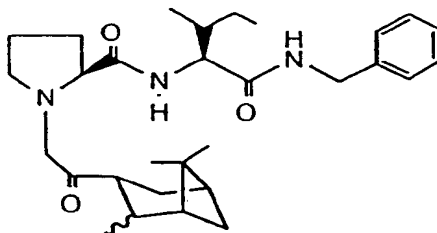
$R_f$  (100% ethyl acetate) = 0.12

HRMS calcd for  $(M+H)^+$  [ $(C_{30}H_{45}N_4O_3 + H)^+$ ] ion 509.7166; found 509.3494

**Example 36**

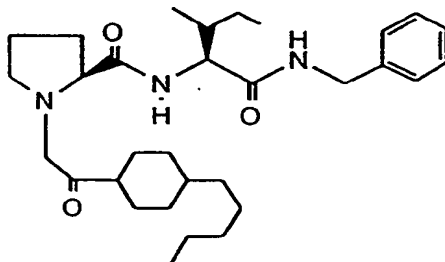
**L-Isoleucine, N-[1-(2-(cis-Octahydro-pentalen-1-yl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, a solution of L-proline-L-isoleucine benzylamide (115 mg, 0.36 mmol), and triethylamine (346  $\mu$ L, 2.5 mmol, 5.0 eq), in THF (2 mL), was treated with 181 mg (0.97 mmol) of 1-chloro-2-(octahydro-pentalen-1-yl)-2-oxoethane (prepared from cis-bicyclo[3.3.0]octane-2-carboxylic acid) to provide 52 mg, (23%) of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

Rf (50% ethyl acetate in dichloromethane) = 0.36

**Example 37**

**L-Isoleucine, N-[1-[2-(2,6,6-Trimethyl-Bicyclo[3.1.1]hept-3-yl)-2-Oxoethyl]-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, a solution of L-proline-L-isoleucine benzylamide (153 mg, 0.48 mmol) and triethylamine (337  $\mu$ L, 5.0 eq), in THF (2 mL), was treated with 205 mg (0.95 mmol) of 1-chloro-2-(2,6,6-trimethyl-bicyclo[3.1.1]hept-3-yl)-2-oxoethane (prepared from (-)-3-pinane-carboxylic acid) to provide 164 mg, (68%) of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

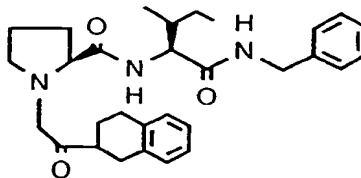
Rf (50% ethyl acetate in dichloromethane) = 0.42

**Example 38**

**L-Isoleucine, N-[1-(2-(4-Pentylcyclohexyl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, a solution of L-proline-L-isoleucine benzylamide (160 mg, 0.50 mmol), and triethylamine (352  $\mu$ L, 2.53 mmol, 5.0 eq), in THF (2 mL), was treated with 232 mg (1.0 mmol) of 1-chloro-2-(4-pentylcyclohexyl)-2-oxoethane (prepared from trans-4-pentylcyclohexanecarboxylic acid) to provide 154 mg (25%) of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

Rf (50% ethyl acetate in dichloromethane) = 0.45

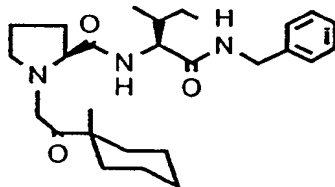
### Example 39



**L-Isoleucine, N-[1-(2-(1,2,3,4-tetrahydro-Naphthalen-2-yl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, a solution of L-proline-L-isoleucine benzylamide (162 mg, 0.51 mmol), and triethylamine (355  $\mu$ L, 5.0 eq), THF (2 mL), was treated with 212 mg (2.0 eq) of 1-chloro-2-(1,2,3,4-tetrahydro-naphthalen-2-yl)-2-oxoethane (prepared from 1,2,3,4-tetrahydro-2-naphthoic acid) to provide 191 mg (77%) of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

Rf (50% ethyl acetate in dichloromethane) = 0.42

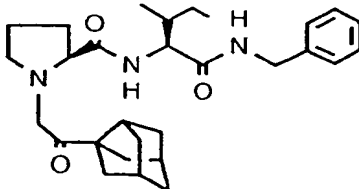
### Example 40



**L-Isoleucine, N-[1-(2-(1-Methyl-Cyclohexyl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, a solution of L-proline-L-isoleucine benzylamide (164 mg, 0.51 mmol), and triethylamine (355  $\mu$ L, 5.0 eq) in THF (2 mL), was treated with 180 mg (2.0 eq) of 1-chloro-2-(1-methyl-cyclohexyl)-2-oxoethane (prepared from 1-methyl-1-cyclohexanecarboxylic acid) to provide 183 mg (93%) of the title compound as a colorless residue. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

Rf (66% ethyl acetate in hexane) = 0.32

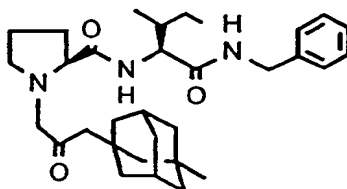
### Example 41



**L-Isoleucine, N-[1-(2-Oxo-2-Tricyclo[3.3.1.0<sup>3,7</sup>]Non-3-yl-Ethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, a solution of L-proline-L-isoleucine benzylamide (167 mg, 0.52 mmol), and triethylamine (367  $\mu$ L, 5.0 eq) in THF (2 mL), was treated with 209 mg (1.2 eq) of 1-chloro-2-oxo-2-tricyclo[3.3.1.0<sup>3,7</sup>]non-3-yl-ethane (prepared from 3-noradamantanecarboxylic acid) to provide 181 mg of the title compound as a colorless residue. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

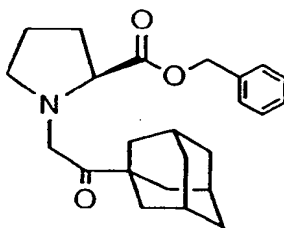
Rf (9% methanol in dichloromethane) = 0.70



**Example 42**

**L-Isoleucine, N-[1-(2-Oxo-3-(3-Methyl-Adamantan-1-yl)-Propyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, a solution of L-proline-L-isoleucine benzylamide (162 mg, 0.51 mmol), and triethylamine (358  $\mu$ L, 5.0 eq), in THF (2 mL), was treated with 246 mg (1.0 eq) of 1-chloro-2-oxo-3-(3-methyl-adamantan-1-yl)-propane (prepared from 3-methyl-1-adamantaneacetic acid) to provide 210 mg (78%) of the title compound as a colorless residue. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

R<sub>f</sub> (50% ethyl acetate in hexane) = 0.43

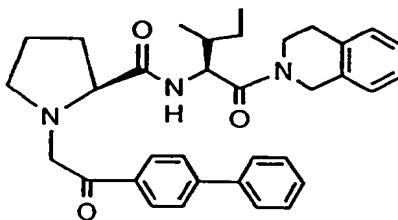
**Example 43**

**L-Proline, 1-(2-Adamantan-1-yl-2-Oxoethyl) Benzyl Ester.** Using the procedure described in example 1e, a solution containing the hydrochloride salt of L-proline benzyl ester (25.46 g, 105.3 mmol), triethylamine (58.0 mL, 414 mmol, 4.0 eq), 1-adamantyl bromomethyl ketone (26.6 g, 103.4 mmol, 1.0 eq) and THF (500 mL), was warmed to reflux, cooled, and purified by flash chromatography to provide 30.0 g (76%) of the title compound as a white foam. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

R<sub>f</sub> (50% dichloromethane in ethyl acetate) = 0.42

**Example 44**

**L-Isoleucine, N-[1-(2-(Biphenyl-4-yl)-2-Oxoethyl)-L-Prolyl] 1,2,3,4-Tetrahydroisoquinolinamide.**



a) **N-[2-(Biphenyl-4-yl)-2-Oxoethyl] L-Proline Benzyl Ester.** Using the procedure described in Example 1e, a solution of the hydrochloride salt of L-proline benzyl ester (20.75 g, 98.0 mmol), was treated with cesium carbonate (87.1 g, 267 mmol, 3.0 eq), 2-bromo-4'-phenylacetophenone (24.5 g, 89.0 mmol, 1.0 eq) in acetonitrile (500 mL), to provide 10.07 g (28%) of L-proline, N-[2-(biphenyl-4-yl)-2-oxoethyl] benzyl ester.

b) **N-[2-(Biphenyl-4-yl)-2-Oxoethyl] L-Proline.** A solution of N-[2-(biphenyl-4-yl)-2-oxoethyl] L-proline benzyl ester in methanol (300 mL) and treated with 10% palladium on carbon (908 mg). Hydrogenation

as described in example 1d, followed by recrystallization from ethyl ether provided 1.72 g (6.2 % overall) of the title compound as a white solid. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

mp = 182-184 °C

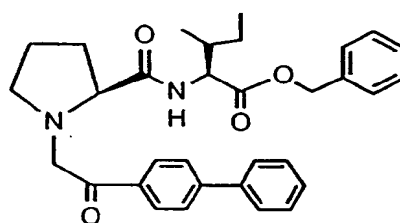
$R_f$  (17% MeOH in dichloromethane) = 0.38

c) L-Isoleucine. N-[1-(2-(Biphenyl-4-yl)-2-Oxoethyl)-L-Prolyl] 1,2,3,4-Tetrahydroisoquinolinamide. A solution of N-[2-(biphenyl-4-yl)-2-oxoethyl] L-proline (50 mg, 0.16 mmol, 1.0 eq), N-ethylmorpholine (125  $\mu\text{L}$ , 0.97 mmol, 6 eq) in acetonitrile (0.5 mL) was cooled to 0 °C and treated with a 50% solution of 1-n-propylphosphonic acid cyclic anhydride in dichloromethane (170  $\mu\text{L}$ , 0.26 mmol, 1.6 eq) followed by the L-isoleucine 1,2,3,4-tetrahydroisoquinolinamide (prepared as described in example 1a; 47.9 mg, 0.19 mmol, 1.2 eq). Purification by HPLC provided 5.53 mg (6.3 %) of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

$R_f$  (50 % ethyl acetate in hexane) = 0.51

HRMS calcd for  $(\text{M} + \text{H})^+$  [ $\text{C}_{34}\text{H}_{40}\text{N}_3\text{O}_3 + \text{H}^+$ ] ion 538.7154; found 538.3072

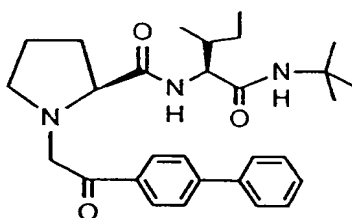
#### Example 45



**L-Isoleucine, N-[1-(2-(Biphenyl-4-yl)-2-Oxoethyl)-L-Prolyl] Benzyl Ester.** A solution of N-[2-(biphenyl-4-yl)-2-oxoethyl] L-proline (102 mg, 0.33 mmol, 1.0 eq), N-ethylmorpholine (270  $\mu\text{L}$ , 2.12 mmol, 6.4 eq) in acetonitrile (1.0 mL) was cooled to 0 °C and treated with a 50% solution of 1-n-propylphosphonic acid cyclic anhydride in dichloromethane (340  $\mu\text{L}$ , 0.53 mmol, 1.6 eq) followed by the tosylate salt of L-isoleucine benzyl ester (143 mg, 0.36 mmol, 1.1 eq). Purification by HPLC provided 45 mg (26%) of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

$R_f$  50 % ethyl acetate in hexane) = 0.29

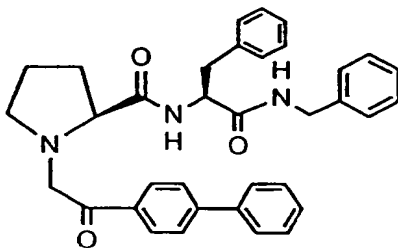
#### Example 46



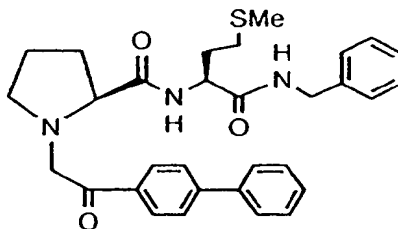
**L-Isoleucine, N-[1-(2-(Biphenyl-4-yl)-2-Oxoethyl)-L-Prolyl] tert-Butylamide.** A solution of N-[2-(biphenyl-4-yl)-2-oxoethyl] L-proline (50 mg, 0.16 mmol, 1.0 eq), N-ethylmorpholine (125  $\mu\text{L}$ , 0.98 mmol, 6 eq) in acetonitrile (0.5 mL) was cooled to 0 °C and treated with a 50% solution of 1-n-propylphosphonic acid cyclic anhydride in dichloromethane (170  $\mu\text{L}$ , 0.27 mmol, 1.6 eq) followed by L-isoleucine tert-butylamide (49 mg, 0.19 mmol, 1.1 eq). Purification by HPLC provided 12 mg (16%) of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

$R_f$  50 % ethyl acetate in hexane) = 0.38

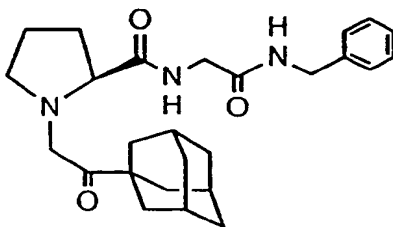
HRMS calcd for  $(\text{M} + \text{H})^+$  [ $\text{C}_{29}\text{H}_{40}\text{N}_3\text{O}_3 + \text{H}^+$ ] ion 478.6604; found 478.3072

**Example 47**

**L-Phenylalanine, N-[1-(2-(Biphenyl-4-yl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** A solution of N-[2-(biphenyl-4-yl)-2-oxoethyl] L-proline (51 mg, 0.16 mmol, 1.0 eq), N-ethylmorpholine (125  $\mu$ L, 0.98 mmol, 6 eq) in acetonitrile (0.5 mL) was cooled to 0  $^{\circ}$ C and treated with a 50% solution of 1-n-propylphosphonic acid cyclic anhydride in dichloromethane (170  $\mu$ L, 0.27 mmol, 1.6 eq) followed by phenylalanine benzylamide (49 mg, 0.19 mmol, 1.1 eq). Purification by HPLC provided 12 mg (14%) of the title compound. The 300 MHz,  $^1$ H NMR analysis of this compound was consistent with the structure. Rf 50 % ethyl acetate in hexane) = 0.38

**Example 48**

**L-Methionine, N-[1-(2-(Biphenyl-4-yl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** A solution of N-[2-(biphenyl-4-yl)-2-oxoethyl] L-proline (54 mg, 0.17 mmol, 1.0 eq), N-ethylmorpholine (133  $\mu$ L, 1.04 mmol, 6 eq) in acetonitrile (0.5 mL) was cooled to 0  $^{\circ}$ C and treated with a 50% solution of 1-n-propylphosphonic acid cyclic anhydride in dichloromethane (180  $\mu$ L, 0.27 mmol, 1.6 eq) followed by the L-methionine benzylamide (71.36 mg, 0.30 mmol, 1.7 eq). Purification by HPLC provided 35 mg (38%) of the title compound. The 300 MHz,  $^1$ H NMR analysis of this compound was consistent with the structure. Rf 50 % ethyl acetate in hexane) = 0.40  
HRMS calcd for  $(M + H)^+$  [ $(C_{31}H_{35}N_3O_3S + H)^+$ ] ion 530.711; found 530.2480

**Example 49**

**Glycine, N-[1-(2-Adamantan-1-yl-2-Ox ethyl)-L-Prolyl] Benzylamide.**

a) 1-(2-Adamantan-1-yl-2-Oxoethyl) L-Proline. Using the procedure described in example 1d, a solution of L-proline, 1-(2-adamantan-1-yl-2-oxoethyl) benzyl ester (30.0 g, 76.63 mmol), 10% palladium on carbon (4.2 g), and methanol (100 mL) was purged with hydrogen, and stirred under an atmosphere of hydrogen until no more benzyl ester was observed by TLC. The solution was purged with argon, filtered

through a plug of celite, and concentrated in vacuo, and recrystallized from ethyl ether to provide 19.45 g, (85%) of the title compound as a white solid. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure.

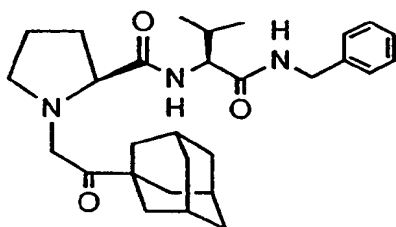
mp 130-142 (sweet), 143-145 (melt)

R<sub>f</sub> (20% MeOH in dichloromethane) = 0.32

b) Glycine, N-[1-(2-Adamantan-1-yl-2-Oxoethyl)-L-Prolyl] Benzylamide. Using the procedure described in example 1a, a solution of 1-(2-adamantan-1-yl-2-oxoethyl) L-proline (104.6 mg, 0.36 mmol, 1.0 eq), triethylamine (150 uL, 1.07 mmol, 3.0 eq), and THF (1.5 mL), was treated with isobutyl chloroformate (51 uL, 0.39 mL, 1.1 eq), then with glycine N-benzylamide (88.2 mg, 537 umol, 1.5 eq). Workup as before provided 53.9 mg (34%) of the title compound as a white foam. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure.

R<sub>f</sub> (50 % ethyl acetate in dichloromethane) = 0.22

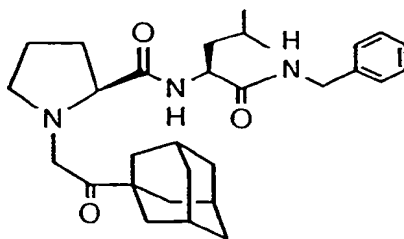
#### Example 50



**L-Valine, N-[1-(2-Adamantan-1-yl-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1a, a solution of 1-(2-adamantan-1-yl-2-oxoethyl) L-proline (109.1 mg, 0.37 mmol, 1.0 eq), triethylamine (156 uL, 1.12 mmol, 3.0 eq), and THF (1.5 mL), was treated with isobutyl chloroformate (53 uL, 0.41 mL, 1.1 eq), then with L-valine benzylamide (117.5 mg, 570 umol, 1.5 eq). Workup as before provided 127 mg (71%) of the title compound as a white foam. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure.

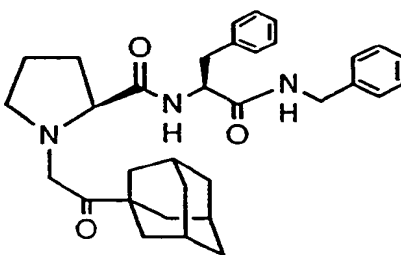
R<sub>f</sub> (50 % ethyl acetate in dichloromethane) = 0.40

#### Example 51

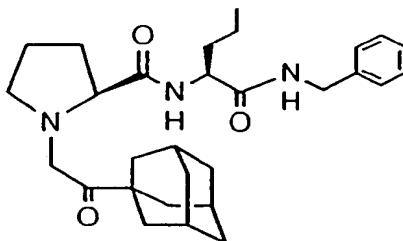


**L-Leucine, N-[1-(2-Adamantan-1-yl-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described previously, a solution of 1-(2-adamantan-1-yl-2-oxoethyl) L-proline (108 mg, 0.37 mmol, 1.0 eq), triethylamine (156 uL, 1.11 mmol, 3.0 eq), and THF (1.5 mL), was treated with isobutyl chloroformate (53 uL, 0.41 mL, 1.1 eq), then with L-leucine N-benzylamide (123 mg, 570 umol, 1.5 eq). Workup as before provided 124 mg (68%) of the title compound. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure.

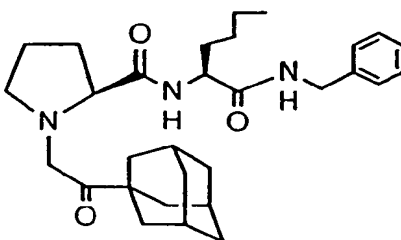
R<sub>f</sub> (50 % ethyl acetate in dichloromethane) = 0.48

**Example 52**

**L-Phenylalanine, N-[1-(2-Adamantan-1-yl-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described previously, a solution of 1-(2-adamantan-1-yl-2-oxoethyl) L-proline (100 mg, 0.34 mmol, 1.0 eq), L-phenylalanine N-benzylamide (175 mg, 686  $\mu$ mol, 2.0 eq) and dichloromethane (1.0 mL), was cooled to 0 °C, and treated with triethylamine (286  $\mu$ L, 2.0 mmol, 6.0 eq). To this chilled solution was added a 50 % solution of 1-n-propylphosphonic acid cyclic anhydride in dichloromethane (436  $\mu$ L, 0.68 mL, 2.0 eq). When TLC indicated the reaction was complete, the solution was allowed to warm to 22 °C, washed with satd aq NaHCO<sub>3</sub>, concentrated in vacuo, and purified by flash chromatography to provide 144 mg (80%) of the title compound. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure. R<sub>f</sub> (50 % ethyl acetate in dichloromethane) = 0.36

**Example 53**

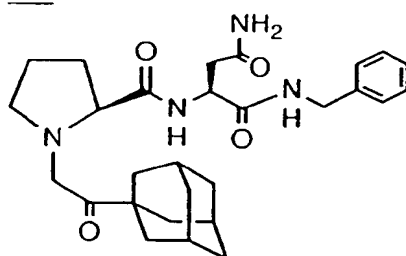
**L-Norvaline, N-[1-(2-Adamantan-1-yl-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described previously, a solution of 1-(2-adamantan-1-yl-2-oxoethyl) L-proline (155 mg, 0.53 mmol, 1.0 eq), L-norvaline N-benzylamide (220 mg, 1.07 mmol, 2.0 eq) and dichloromethane (1.0 mL), was treated with triethylamine (446  $\mu$ L, 3.2 mmol, 6.0 eq), and a 50 % solution of 1-n-propylphosphonic acid cyclic anhydride in dichloromethane (680  $\mu$ L, 1.07 mL, 2.0 eq), to provide 372 mg (70%) of the title compound. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure. R<sub>f</sub> (50 % ethyl acetate in dichloromethane) = 0.37

**Example 54**

**L-Norleucine, N-[1-(2-Adamantan-1-yl-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described previously, a solution of 1-(2-adamantan-1-yl-2-oxoethyl) L-proline (154 mg, 0.53 mmol, 1.0 eq), L-norleucine N-benzylamide (234 mg, 1.07 mmol, 2.0 eq) and dichloromethane (1.0 mL), was treated with triethylamine (446  $\mu$ L, 3.2 mmol, 6.0 eq), and a 50 % solution of 1-n-propylphosphonic acid cyclic

anhydride in dichloromethane (680  $\mu$ L, 1.07 mL, 2.0 eq), to provide 210 mg (80%) of the title compound as a white foam. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.  
 $R_f$  (50 % ethyl acetate in dichloromethane) = 0.44

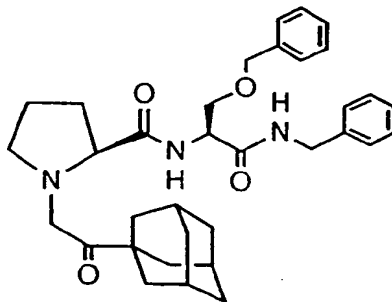
#### Example 55



**L-Asparagine, N-[1-(2-Adamantan-1-yl-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described previously, a heterogeneous solution of 1-(2-adamantan-1-yl-2-oxoethyl) L-proline (152 mg, 0.52 mmol, 1.0 eq), L-asparagine N-benzylamide (231 mg, 1.04 mmol, 2.0 eq) and dichloromethane (1.5 mL), was treated with triethylamine (436  $\mu$ L, 3.1 mmol, 6.0 eq), and a 50 % solution of 1-n-propylphosphonic acid cyclic anhydride in dichloromethane (663  $\mu$ L, 1.04 mL, 2.0 eq), to provide 130.7 mg (51%) of the title compound as a white foam. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

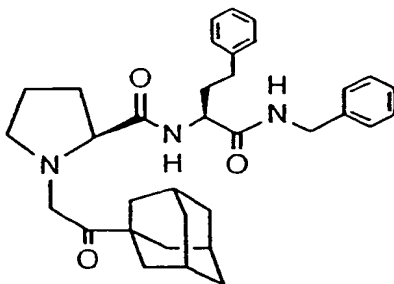
$R_f$  (10% MeOH in dichloromethane) = 0.41

#### Example 56



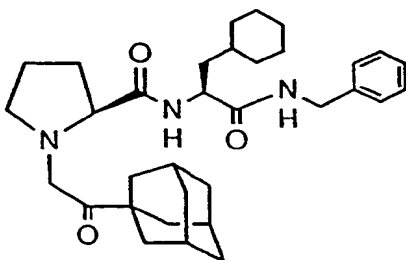
**L-Serine-(O-Benzyl Ether), N-[1-(2-Adamantan-1-yl-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described previously, a solution of 1-(2-adamantan-1-yl-2-oxoethyl) L-proline (151 mg, 0.52 mmol, 1.0 eq), L-serine-(O-benzyl ether)-N-benzylamide (294 mg, 1.03 mmol, 2.0 eq) and dichloromethane (1.5 mL), was treated with triethylamine (432  $\mu$ L, 3.1 mmol, 6.0 eq), and a 50 % solution of 1-n-propylphosphonic acid cyclic anhydride in dichloromethane (658  $\mu$ L, 2.0 eq), to provide 220 mg (76%) of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

$R_f$  (50% ethyl acetate in dichloromethane) = 0.30

**Example 57**

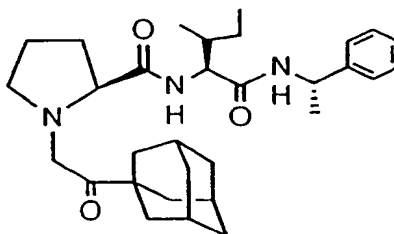
**L-β-Phenylalanine, N-[1-(2-Adamantan-1-yl-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described previously, a solution of 1-(2-adamantan-1-yl-2-oxoethyl) L-proline (154 mg, 0.53 mmol, 1.0 eq), L-β-phenylalanine N-benzylamide (286 mg, 1.06 mmol, 2.0 eq) and dichloromethane (1.5 mL), was treated with triethylamine (442 uL, 3.2 mmol, 6.0 eq), and a 50 % solution of 1-n-propylphosphonic acid cyclic anhydride in dichloromethane (673 uL, 2.0 eq), to provide 229 mg (81%) of the title compound. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure.

R<sub>f</sub> (50% ethyl acetate in dichloromethane) = 0.40

**Example 58**

**L-Cyclohexylalanine, N-[1-(2-Adamantan-1-yl-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described previously, a solution of 1-(2-adamantan-1-yl-2-oxoethyl) L-proline (155 mg, 0.53 mmol, 1.0 eq), L-cyclohexylalanine N-benzylamide (248 mg) and dichloromethane (1.5 mL), was treated with triethylamine (444 uL, 3.2 mmol, 6.0 eq), and a 50 % solution of 1-n-propylphosphonic acid cyclic anhydride in dichloromethane (676 uL, 2.0 eq), to provide 148 mg (52%) of the title compound. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure.

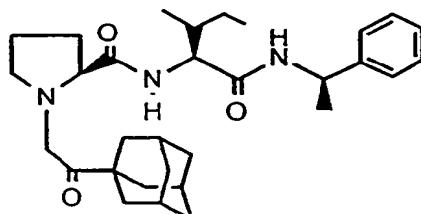
R<sub>f</sub> (50% ethyl acetate in dichloromethane) = 0.42

**Example 59**

**L-Isoleucine, N-[1-(2-Adamantan-1-yl-2-Oxoethyl)-L-Prolyl] alpha-(S)-methylbenzylamide.** Using the procedure described previously, a solution of 1-(2-adamantan-1-yl-2-oxoethyl) L-proline (154 mg, 0.53 mmol, 1.0 eq), L-isoleucine alpha-(S)-methylbenzylamide (247 mg, 1.05 mmol) and dichloromethane (1.5 mL), was treated with triethylamine (441 uL, 3.2 mmol, 6.0 eq), and a 50 % solution of 1-n-propyl-

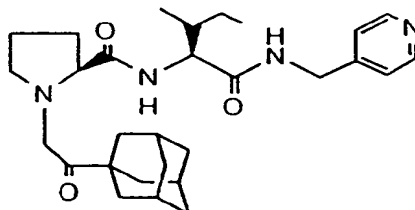
phosphonic acid cyclic anhydride in dichloromethane (671  $\mu$ L, 2.0 eq), to provide 192 mg (72%) of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.  
 $R_f$  (50% ethyl acetate in dichloromethane) = 0.44

#### Example 60



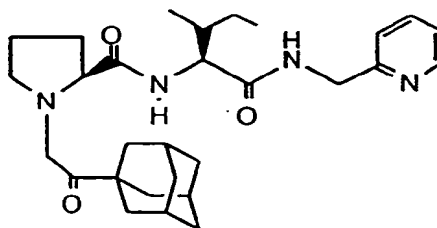
**L-isoleucine, N-[1-(2-Adamantan-1-yl-2-Oxoethyl)-L-Prolyl] alpha-(R)-methylbenzylamide.** Using the procedure described previously, a solution of 1-(2-adamantan-1-yl-2-oxoethyl) L-proline (146 mg, 0.53 mmol, 1.0 eq), L-isoleucine alpha-(R)-methylbenzylamide (234 mg, 1.05 mmol) and dichloromethane (1.5 mL), was treated with triethylamine (420  $\mu$ L, 6.0 eq), and a 50 % solution of 1-n-propylphosphonic acid cyclic anhydride in dichloromethane (638  $\mu$ L, 2.0 eq), to provide 108 mg (43%) of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.  
 $R_f$  (15% methanol in dichloromethane) = 0.47

#### Example 61



**L-isoleucine, N-[1-(2-Adamantan-1-yl-2-Oxoethyl)-L-Prolyl] Pyridin-4-ylmethylamide.** Using the procedure described previously, a solution of 1-(2-adamantan-1-yl-2-oxoethyl) L-proline (230 mg, 0.78 mmol, 1.0 eq), L-isoleucine pyridin-4-ylmethylamide (266 mg, 1.5 mmol) and dichloromethane (2 mL), was treated with triethylamine (660  $\mu$ L, 6.0 eq), and a 50 % solution of 1-n-propylphosphonic acid cyclic anhydride in dichloromethane (1.0 mL, 2.0 eq), to provide 180 mg (46%) of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.  
 $R_f$  (4% methanol in dichloromethane) = 0.23

#### Example 62



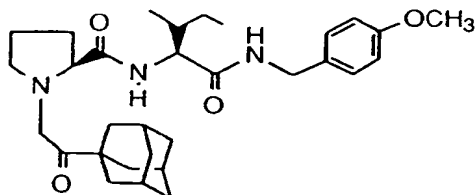
**L-isoleucine, N-[1-(2-Adamantan-1-yl-2-Oxoethyl)-L-Prolyl] Pyridin-2-ylmethylamide.** Using the procedure described previously, a solution of 1-(2-adamantan-1-yl-2-oxoethyl) L-proline (165 mg, 0.56 mmol, 1.0 eq), L-isoleucine pyridin-2-ylmethylamide (246 mg, 1.11 mmol) and dichloromethane (2 mL), was treated with triethylamine (475  $\mu$ L, 6.0 eq), and a 50 % solution of 1-n-propylphosphonic acid cyclic anhydride in



dichloromethane (721  $\mu$ L, 2.0 eq), to provide 214 mg (77%) of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

$R_f$  (5% methanol in dichloromethane) = 0.21

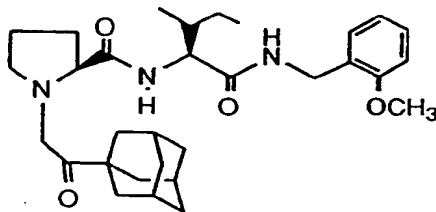
### 5 Example 63



15 **L-Isoleucine, N-[1-(2-Adamantan-1-yl-2-Oxoethyl)-L-Prolyl] 4-methoxybenzylamide.** Using the procedure described previously, a solution of 1-(2-adamantan-1-yl-2-oxoethyl) L-proline (140 mg, 0.48 mmol, 1.0 eq), L-isoleucine 4-methoxybenzylamide (238 mg, 0.95 mmol) and dichloromethane (2 mL), was treated with triethylamine (400  $\mu$ L, 6.0 eq), and a 50 % solution of 1-n-propylphosphonic acid cyclic anhydride in dichloromethane (610  $\mu$ L, 2.0 eq), to provide 207 mg (82%) of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

20  $R_f$  (50% ethyl acetate in dichloromethane) = 0.31

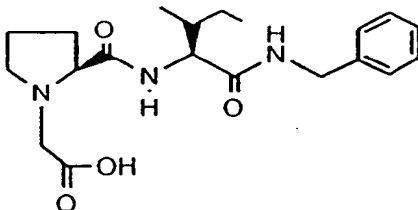
### Example 64



25 **L-Isoleucine, N-[1-(2-Adamantan-1-yl-2-Oxoethyl)-L-Prolyl] 2-methoxybenzylamide.** Using the procedure described previously, a solution of 1-(2-adamantan-1-yl-2-oxoethyl) L-proline (179 mg, 0.61 mmol, 1.0 eq), L-isoleucine 2-methoxybenzylamide (308 mg, 1.23 mmol) and dichloromethane (2 mL), was treated with triethylamine (515  $\mu$ L, 6.0 eq), and a 50 % solution of 1-n-propylphosphonic acid cyclic anhydride in dichloromethane (783  $\mu$ L, 2.0 eq), to provide 254 mg (79%) of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

30  $R_f$  (50% ethyl acetate in dichloromethane) = 0.31

### Example 65



45 **L-Isoleucine, N-[1-(Carboxymethyl)-L-Prolyl] Benzylamide.** Using the hydrogenation conditions described in example 1d, a solution of L-isoleucine, N-[1-(2-benzyloxy-2-oxoethyl)-L-prolyl] benzylamide (14.80 g, 31.76 mmol), 10% palladium on carbon (0.80 g), and methanol (350 mL), was purged with hydrogen, and stirred under an atmosphere of hydrogen at 22  $^{\circ}\text{C}$ . After 8 hrs, the solution was purged with argon, filtered through a plug of celite, and concentrated in vacuo to provide 11.47 g (96%) of the title compound as a white solid. The 300 MHz,  $^1\text{H}$  NMR and mass spectrum analysis of this compound was

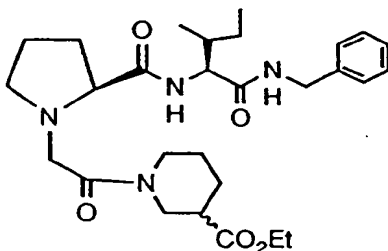
consistent with the structure.

mp = 76-80 °C

R<sub>f</sub> (100% ethyl acetate) = 0.04

Mass Spectrum (EI) m/e (rel intensity) 376 (20, M + H), 307 (38), 154 (100), 136 (82).

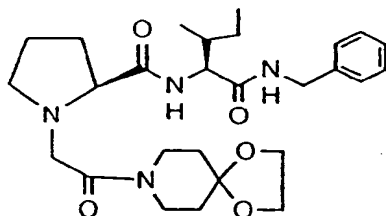
#### Example 66



**L-Isoleucine, N-[1-[2-[N-(Piperidine-3-Carboxylic Acid Ethyl Ester)]-2-Oxoethyl]-L-Prolyl] Benzylamide.** A -5 °C solution of L-isoleucine, N-[1-(carboxymethyl)-L-prolyl] benzylamide (266 mg, 0.71 mmol, 1.0 eq), N-ethylmorpholine (135 uL, 1.06 mmol, 1.5 eq) in acetonitrile (5.0 mL) was treated with isobutyl chloroformate (101 uL, 0.78 mmol, 1.1 eq) followed by ethyl nipecotate (220 uL, 1.40 mmol, 2.0 eq). Purification by flash chromatography provided 150 mg (41%) of the title compound as a colorless oil. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure.

R<sub>f</sub> (100% ethyl acetate) = 0.22

#### Example 67

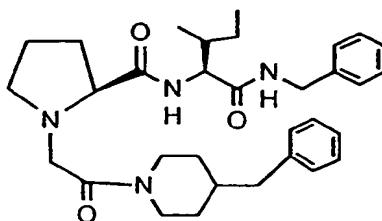


**L-Isoleucine, N-[1-(2-(1,4-Dioxo-8-aza-spiro[4.5]dec-8-yl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** A -5 °C solution of L-isoleucine, N-[1-(carboxymethyl)-L-prolyl] benzylamide (254 mg, 0.67 mmol, 1.0 eq), N-methylmorpholine (96 uL, 0.88 mmol, 1.30 eq) in acetonitrile (7 mL) was treated with isobutyl chloroformate (96 uL, 0.74 mmol, 1.1 eq) followed by 1,4-dioxo-8-aza-spiro[4.5] decane (0.193 g, 1.35 mmol, 2.0 eq). Purification by flash chromatography provided 280 mg (83%) of the title compound as a colorless oil. The 300 MHz, <sup>1</sup>H NMR and mass spectrum analysis of this compound was consistent with the structure.

R<sub>f</sub> (100% ethyl acetate) = 0.13

R<sub>f</sub> (20% MeOH in ethyl acetate) = 0.68

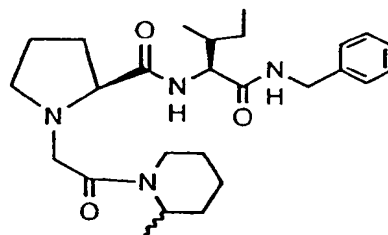
Mass Spectrum (+EI) m/e (rel intensity) 500 (10, M +), 330 (12), 253 (100), 142 (18).

**Example 68**

**L-Isoleucine, N-[1-[2-(N-(4-Benzylpiperidyl))-2-Oxoethyl]-L-Prolyl] Benzylamide.** A -5 °C solution of L-isoleucine, N-[1-(carboxymethyl)-L-prolyl] benzylamide (321 mg, 0.85 mmol, 1.0 eq), N-methylmorpholine (122 uL, 1.11 mmol, 1.30 eq) in acetonitrile (8 mL) was treated with isobutyl chloroformate (127 uL, 0.98 mmol, 1.15 eq) followed by 4-benzylpiperidine (0.299 g, 1.71 mmol, 2.0 eq). Purification by flash chromatography provided 401 mg (88%) of the title compound as a colorless oil. The 300 MHz, <sup>1</sup>H NMR and mass spectrum analysis of this compound was consistent with the structure.

R<sub>f</sub> (100% ethyl acetate) = 0.29; R<sub>f</sub> (20% MeOH in ethyl acetate) = 0.69

Mass Spectrum (+ Cl) m/e (rel intensity) 532 (70, M<sup>+</sup>), 425 (30), 357 (22), 330 (100).

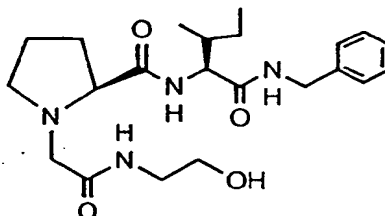
**Example 69**

**L-Isoleucine, N-[1-[2-(2-Methylpiperidine)-2-Oxoethyl]-L-Prolyl] Benzylamide.** A -5 °C solution of L-isoleucine, N-[1-(carboxymethyl)-L-prolyl] benzylamide (202 mg, 0.53 mmol, 1.0 eq), N-ethylmorpholine (82 uL, 0.64 mmol, 1.20 eq) in acetonitrile (8 mL) was treated with isobutyl chloroformate (80 uL, 0.62 mmol, 1.15 eq) followed by 2-methylpiperidine (0.106 g, 1.07 mmol, 2.0 eq). The solution was warmed to 22 °C, concentrated to a residue, and purified by flash chromatography to provide 80 mg (32%) of the title compound as a colorless oil. The 300 MHz, <sup>1</sup>H NMR and mass spectrum analysis of this compound was consistent with the structure.

R<sub>f</sub> (100% ethyl acetate) = 0.26

R<sub>f</sub> (20% MeOH in ethyl acetate) = 0.61

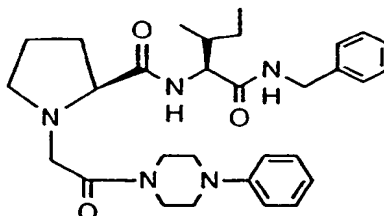
Mass Spectrum (+ EI) m/e (rel intensity) 456 (10, M<sup>+</sup>), 330 (14), 209 (100), 82 (82)

**Example 70**

**L-Isoleucine, N-[1-(2-(2-Hydroxyethylamine)-2-Ox ethyl)-L-Prolyl] Benzylamid .** A -5 °C solution of L-isoleucine, N-[1-(carboxymethyl)-L-prolyl] benzylamide (0.215 g, 0.58 mmol, 1.0 eq), and triethylamine (96 uL, 70 mg, 0.64 mmol, 1.20 eq), in acetonitrile (10 mL) was treated with isobutyl chloroformate (83 uL, 86

mg, 0.63 mmol, 1.1 eq) followed by 2-aminoethanol (69  $\mu$ L, 70 mg, 1.15 mmol, 2.0 eq). Workup as above provided 127 mg (53 %) of the title compound. The 300 MHz,  $^1\text{H}$  NMR and mass spectrum analysis of this compound was consistent with the structure.  $R_f(100\%$  ethyl acetate) = 0.05  
Mass Spectrum (+ EI) m/e (rel intensity) 418 (10, M+), 330 (10), 171 (100), 153 (30).

#### Example 71

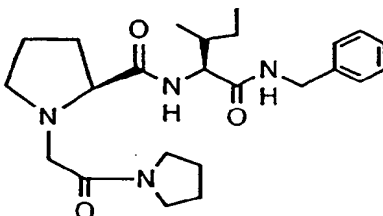


**L-Isoleucine, N-[1-[2-(4-Phenylpiperazine)-2-Oxoethyl]-L-Prolyl] Benzylamide.** A -5  $^{\circ}\text{C}$  solution of L-isoleucine, N-[1-(carboxymethyl)-L-prolyl] benzylamide (0.214 g, 0.58 mmol, 1.0 eq), and triethylamine (96  $\mu$ L, 70 mg, 0.64 mmol, 1.20 eq), in acetonitrile (6 mL) was treated with isobutyl chloroformate (83  $\mu$ L, 86 mg, 0.63 mmol, 1.1 eq) followed by 4-phenylpiperazine (170  $\mu$ L, 186 mg, 1.15 mmol, 2.0 eq). Workup as above provided 170 mg (57 %) of the title compound. The 300 MHz,  $^1\text{H}$  NMR and mass spectrum analysis of this compound was consistent with the structure.

$R_f(100\%$  ethyl acetate) = 0.18

Mass Spectrum (+ EI) m/e (rel intensity) 519 (30, M+), 330 (22), 272 (100), 161 (20), 136 (25).

#### Example 72

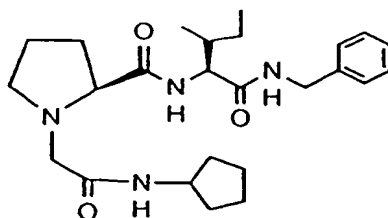


**L-Isoleucine, N-[1-[2-(1-Pyrrolidine)-2-Oxoethyl]-L-Prolyl] Benzylamide.** A -5  $^{\circ}\text{C}$  solution of L-isoleucine, N-[1-(carboxymethyl)-L-prolyl] benzylamide (0.212 g, 0.56 mmol, 1.0 eq), and triethylamine (96  $\mu$ L, 65 mg, 0.65 mmol, 1.15 eq), in acetonitrile (10 mL) was treated with isobutyl chloroformate (77  $\mu$ L, 80 mg, 0.59 mmol, 1.05 eq) followed by pyrrolidine (94  $\mu$ L, 80 mg, 1.13 mmol, 2.0 eq). Workup as above provided 205 mg (85 %) of the title compound. The 300 MHz,  $^1\text{H}$  NMR and mass spectrum analysis of this compound was consistent with the structure.

$R_f(100\%$  ethyl acetate) = 0.14

Mass Spectrum (+ EI) m/e (rel intensity) 428 (13, M+), 330 (10), 208 (10), 181 (100), 82 (85).

#### Example 73



**L-Isoleucine, N-[1-[2-(N-Cyclopentylamino)-2-Oxoethyl]-L-Prolyl] Benzylamide.** A -5  $^{\circ}\text{C}$  solution of L-isoleucine, N-[1-(carboxymethyl)-L-prolyl] benzylamide (0.212 g, 0.56 mmol, 1.0 eq), and triethylamine (96

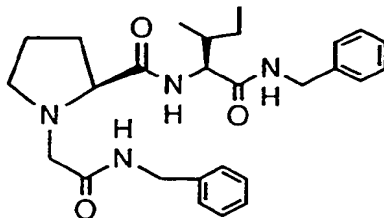
uL, 65 mg, 0.65 mmol, 1.15 eq), in acetonitrile (10 mL) was treated with isobutyl chloroformate (77 uL, 86 mg, 0.59 mmol, 1.05 eq) followed by cyclopentylamine (111 uL, 96 mg, 1.13 mmol, 2.0 eq). Workup as above provided 198 mg (79 %) of the title compound. The 300 MHz, <sup>1</sup>H NMR and mass spectrum analysis of this compound was consistent with the structure.

$R_f$ (100% ethyl acetate) = 0.22

$R_f$ (20% MeOH in ethyl acetate) = 0.55

Mass Spectrum (+ EI) m/e (rel intensity) 442 (28, M<sup>+</sup>), 375 (11), 330 (35), 297 (28), 212 (100).

#### Example 74

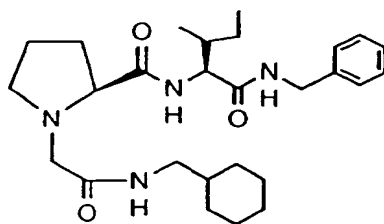


**L-Isoleucine, N-[1-[2-(N-(Phenylmethylamino))-2-Oxoethyl]-L-Prolyl] Benzylamide.** A -5 °C solution of L-isoleucine, N-[1-(carboxymethyl)-L-prolyl] benzylamide (0.218 g, 0.58 mmol, 1.0 eq), and triethylamine (89 uL, 64 mg, 0.64 mmol, 1.10 eq), in acetonitrile (10 mL) was treated with isobutyl chloroformate (79 uL, 83 mg, 0.61 mmol, 1.05 eq) followed by benzylamine (82 uL, 80 mg, 0.75 mmol, 1.3 eq). After the addition was complete, the flask was removed from the cold bath and the solution was stirred at 22 °C for 3 hrs. Workup as above provide 227 mg (84%) of the title compound as a white solid. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure.

mp = 76-80 °C

$R_f$ (100% ethyl acetate) = 0.25

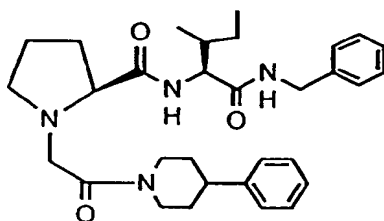
#### Example 75



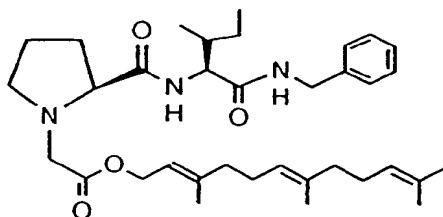
**L-Isoleucine, N-[1-[2-(N-(Cyclohexylmethylamino))-2-Oxoethyl]-L-Prolyl] Benzylamide.** Using the procedure described previously, a solution of L-isoleucine, N-[1-(carboxymethyl)-L-prolyl] benzylamide (0.220 g, 0.58 mmol, 1.0 eq), and triethylamine (89 uL, 64 mg, 0.64 mmol, 1.10 eq), in acetonitrile (10 mL) was treated with isobutyl chloroformate (79 uL, 83 mg, 0.61 mmol, 1.05 eq) followed by cyclohexylmethylamine (99 uL, 86 mg, 0.75 mmol, 1.30 eq). Workup as above provided 201 mg (73 %) of the title compound. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure.

$R_f$ (100% ethyl acetate) = 0.23

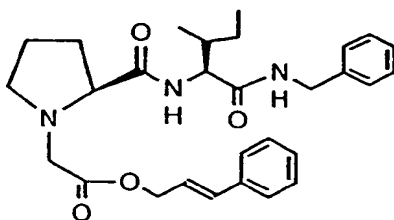
$R_f$ (20% MeOH in ethyl acetate) = 0.64

**Example 76**

**L-Isoleucine, N-[1-(2-(4-Phenylpiperidyl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described above, a solution of L-isoleucine, N-[1-(carboxymethyl)-L-prolyl] benzylamide (0.124 g, 0.33 mmol, 1.0 eq), and N-methylmorpholine (43  $\mu$ L, 40 mg, 0.40 mmol, 1.20 eq), in acetonitrile (3 mL) was treated with isobutyl chloroformate (45  $\mu$ L, 47 mg, 0.35 mmol, 1.05 eq) followed by 4-phenylpiperidine (69 mg, 0.43 mmol, 1.3 eq). Workup as above provided 101 mg (59 %) of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.  
 $R_f$ (100% ethyl acetate) = 0.18

**Example 77**

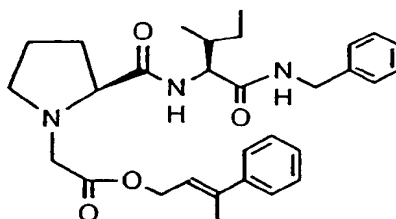
**L-Isoleucine, N-[1-(2-[1-(3,7,11-Trimethyldodeca-2,6,10-trien-1-ol)]-2-Oxoethyl)-L-Proline] Benzylamide.** A solution of L-isoleucine, N-[1-(carboxymethyl)L-prolyl] benzylamide (67 mg, 0.18 mmol), 4-N,N-dimethylaminopyridine (6.1 mg, 0.05 mmol, 0.3 eq), 1,3-dicyclohexylcarbodiimide (64 mg, 0.31 mmol, 1.8 eq) in dichloromethane (2.0 mL) was treated with trans,trans farnesol (55  $\mu$ L, 0.22 mmol, 1.2 eq). After TLC indicated the reaction was complete, the mixture was purified by HPLC to provide 27 mg (26%) of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.  
 $R_f$  (50% ethyl acetate in dichloromethane) = 0.50  
 HRMS calcd for  $(\text{M} + \text{H})^+$   $[(\text{C}_{35}\text{H}_{53}\text{N}_3\text{O}_4 + \text{H})^+]$  ion 580.8365; found 580.4117

**Example 78**

**L-Isoleucine, N-[1-(2-(3-Phenyl-2-Propen-1-Oxy)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described previously, a solution of L-isoleucine, N-[1-(carboxymethyl)-L-prolyl] benzylamide (65 mg, 0.17 mmol), 4-N,N-dimethylaminopyridine (12.5 mg, 0.10 mmol, 0.6 eq), 1,3-dicyclohexylcarbodiimide (64 mg, 0.31 mmol, 1.8 eq) in dichloromethane (5.0 mL) was treated with trans cinnamyl alcohol (29  $\mu$ L, 0.22 mmol, 1.3 eq). After TLC indicated the reaction was complete, the mixture was purified by HPLC to provide 32 mg (38%) of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.  
 $R_f$  (50% ethyl acetate in dichloromethane) = 0.47

HRMS calcd for  $(M + H)^+$   $[(C_{29}H_{38}N_3O_4 + H)^+]$  ion 492.6424; found 492.2864

### Example 79

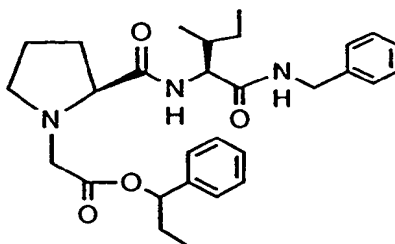


**L-isoleucine, N-[1-(2-(3-Phenyl-3-Methyl-2-Propen-1-Oxy)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described previously, a solution of L-isoleucine, N-[1-(carboxymethyl)-L-prolyl] benzylamide (56 mg, 0.15 mmol), 4-N,N-dimethylaminopyridine (3.6 mg, 0.03 mmol, 0.2 eq), 1,3-dicyclohexylcarbodiimide (54 mg, 0.26 mmol, 1.7 eq) in dichloromethane (5.0 mL) was treated with trans 2-methyl-3-phenyl-2-propen-1-ol (28  $\mu$ L, 0.19 mmol, 1.3 eq). After TLC indicated the reaction was complete, the mixture was purified by HPLC to provide 32 mg (42%) of the title compound. The 300 MHz,  $^1H$  NMR analysis of this compound was consistent with the structure.

$R_f$  (50% ethyl acetate in dichloromethane) = 0.52

HRMS calcd for  $(M + H)^+$   $[(C_{30}H_{40}N_3O_4 + H)^+]$  ion 506.6695; found 506.3021

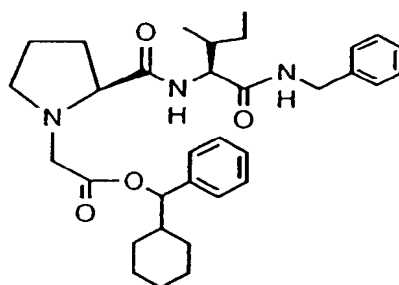
### Example 80



**L-isoleucine, N-[1-(2-(1-Phenylpropoxy)-2-Oxoethyl)-L-Prolyl] Benzylamide.** A solution of L-isoleucine, N-[1-(carboxymethyl)-L-prolyl] benzylamide (59 mg, 0.16 mmol), 4-N,N-dimethylaminopyridine (7.9 mg, 0.06 mmol, 0.4 eq), 1,3-dicyclohexylcarbodiimide (49 mg, 0.23 mmol, 1.5 eq) in dichloromethane (5.0 mL) was treated with (+/-) 1-phenyl-1-propanol (28  $\mu$ L, 0.20 mmol, 1.3 eq). After TLC indicated the reaction was complete, the mixture was purified by HPLC to provide 32 mg (41%) of the title compound. The 300 MHz,  $^1H$  NMR analysis of this compound was consistent with the structure.

$R_f$  (50% ethyl acetate in dichloromethane) = 0.51

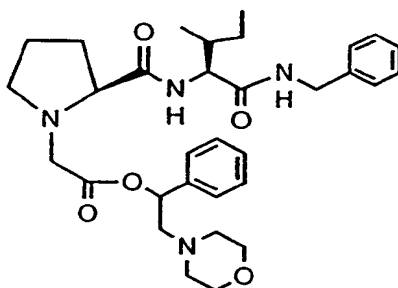
HRMS calcd for  $(M + H)^+$   $[(C_{29}H_{40}N_3O_4 + H)^+]$  ion 494.6584; found 494.3021

**Example 81**

**L-Isoleucine, N-[1-(2-(1-Phenyl-1-Cyclohexylmethoxy)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described previously, a solution of L-isoleucine, N-[1-(carboxymethyl)-L-prolyl] benzylamide (71 mg, 0.19 mmol), 4-N,N-dimethylaminopyridine (7.5 mg, 0.06 mmol, 0.3 eq), 1,3-dicyclohexylcarbodiimide (64 mg, 0.31 mmol, 1.6 eq) in dichloromethane (5.0 mL) was treated with (+/-) cyclohexylphenylcarbinol (45 mg, 0.24 mmol, 1.3 eq). After TLC indicated the reaction was complete, the mixture was purified by HPLC to provide 27 mg (26%) of the title compound. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure.

$R_f$  (50% ethyl acetate in dichloromethane) = 0.51

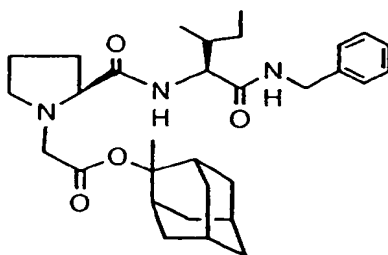
HRMS calcd for  $(M+H)^+$  [ $(C_{33}H_{46}N_3O_3 + H)^+$ ] ion 548.7506; found 548.3491

**Example 82**

**L-Isoleucine, N-[1-(2-(1-Phenyl-2-(4-Morpholino)ethoxy)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described previously, a solution of L-isoleucine, N-[1-(carboxymethyl)-L-prolyl] benzylamide (58 mg, 0.15 mmol), 4-N,N-dimethylaminopyridine (13.2 mg, 0.10 mmol, 0.7 eq), 1,3-dicyclohexylcarbodiimide (57 mg, 0.27 mmol, 1.8 eq) in dichloromethane (5.0 mL) was treated with (+/-) alpha-phenyl-4-morpholinoethanol (60 mg, 0.29 mmol, 1.9 eq). After TLC indicated the reaction was complete, the mixture was purified by HPLC to provide 20 mg (22%) of the title compound. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure.

$R_f$  (50% ethyl acetate in dichloromethane) = 0.32

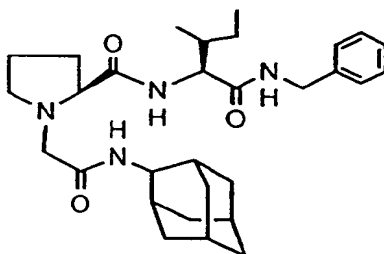


**Example 83**

**L-Isoleucine, N-[1-(2-(2-Oxy-2-Methyladamant-2-yl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described previously, a solution of L-isoleucine, N-[1-(carboxymethyl)-L-prolyl] benzylamide (61 mg, 0.16 mmol), 4-N,N-dimethylaminopyridine (26 mg, 0.21 mmol, 1.3 eq), 1,3-dicyclohexylcarbodiimide (51 mg, 0.25 mmol, 1.5 eq) in dichloromethane (3.0 mL) was treated with 2-methyl-2-adamantanol (33 mg, 0.21 mmol, 1.3 eq). After TLC indicated the reaction was complete, the mixture was purified by HPLC to provide 20 mg (23%) of the title compound. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure.

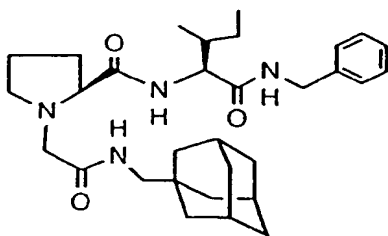
R<sub>f</sub> (50% ethyl acetate in dichloromethane) = 0.45

HRMS calcd for (M + H)<sup>+</sup> [(C<sub>31</sub>H<sub>46</sub>N<sub>3</sub>O<sub>3</sub> + H)<sup>+</sup>] ion 524.7284; found 524.3491

**Example 84**

**L-Isoleucine, N-[1-(Adamantan-2-ylcarbamoylmethyl)-L-Prolyl] Benzylamide.** Using the procedure described previously, a solution of L-isoleucine, N-[1-(carboxymethyl)-L-prolyl] benzylamide (113 mg, 0.30 mmol), triethylamine (168 uL, 1.20 mmol, 4 eq) in acetonitrile (1.5 mL), was cooled to 0 °C and treated with isobutyl chloroformate (43 uL, 0.33 mmol, 1.1 eq) followed by the hydrochloride salt of 2-adamantylamine (113.2 mg, 0.60 mmol, 2 eq). After TLC indicated the reaction was complete, the mixture was purified by HPLC to provide 10 mg (6.5%) of the title compound. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure.

R<sub>f</sub> (100% ethyl acetate) = 0.26

**Example 85**

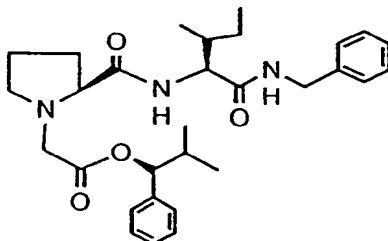
**L-Isoleucine, N-[1-(Adamant-1-ylmethylcarbamoylmethyl)-L-Prolyl] Benzylamide.** Using the procedure described previously, a solution of L-isoleucine, N-[1-(carboxymethyl)-L-prolyl] benzylamide (106 mg, 0.28 mmol), triethylamine (158 uL, 1.13 mmol, 4 eq) in acetonitrile (1.5 mL), was cooled to 0 °C and treated with

isobutyl chloroformate (40.5  $\mu$ L, 0.31 mmol, 1.1 eq) followed by 1-adamantanemethylamine (100  $\mu$ L, 0.57 mmol, 2 eq). After TLC indicated the reaction was complete, the mixture was purified by HPLC to provide 15 mg (10%) of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

5  $R_f$  (100% ethyl acetate) = 0.25

HRMS calcd for  $(\text{M} + \text{H})^+ [(\text{C}_{31}\text{H}_{47}\text{N}_4\text{O}_3 + \text{H})^+]$  ion 523.7436; found 523.3651

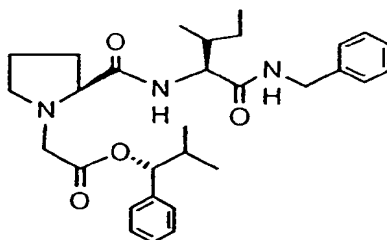
#### Example 86



20 **L-Isoleucine, N-[1-(2-(2-Methyl-1-(S)-Phenyl-1-Propoxy)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described previously, a solution of L-isoleucine, N-[1-(carboxymethyl)-L-prolyl] benzylamide (298 mg, 0.73 mmol), 4-N,N-dimethylaminopyridine (95 mg, 0.77 mmol, 1.2 eq), 1,3-dicyclohexylcarbodiimide (243 mg, 1.18 mmol, 1.8 eq) in dichloromethane (10 mL) was treated with (S)-2-methyl-1-phenyl-1-propanol (100 mg, 0.64 mmol, 1.3 eq). After TLC indicated the reaction was complete, the mixture was purified by HPLC to provide 104 mg (32%) of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

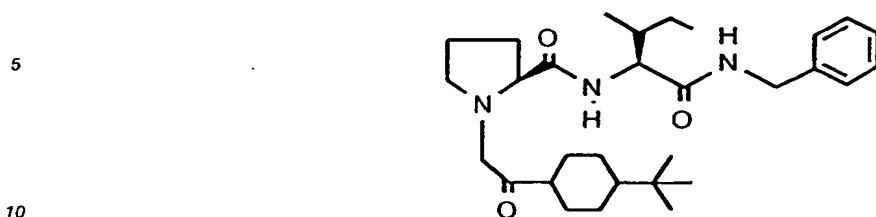
25  $R_f$  (50% ethyl acetate in dichloromethane) = 0.57

#### Example 87



40 **L-Isoleucine, N-[1-(2-(2-Methyl-1-(R)-Phenyl-1-Propoxy)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described previously, a solution of L-isoleucine, N-[1-(carboxymethyl)-L-prolyl] benzylamide (298 mg, 0.73 mmol), 4-N,N-dimethylaminopyridine (100 mg, 0.82 mmol, 1.3 eq), 1,3-dicyclohexylcarbodiimide (242 mg, 1.17 mmol, 1.8 eq) in dichloromethane (10 mL) was treated with (R)-2-methyl-1-phenyl-1-propanol (100 mg, 0.64 mmol, 1.3 eq). After TLC indicated the reaction was complete, the mixture was purified by HPLC to provide 96.2 mg (30%) of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

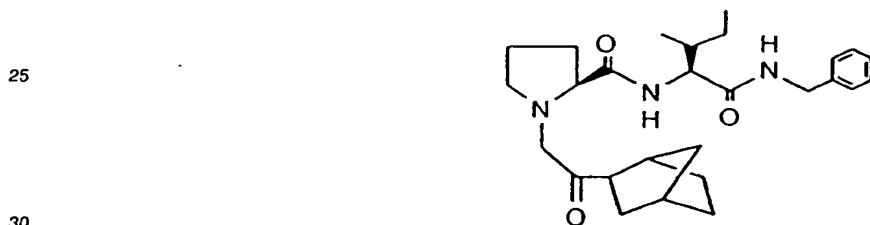
45  $R_f$  (50% ethyl acetate in dichloromethane) = 0.57

**Example 88**

**L-Isoleucine, N-[1-(2-(4-tert-Butylcyclohexyl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of L-proline-L-isoleucine benzylamide (200 mg, 0.63 mmol) with 4-(t-butyl)cyclohexyl  $\alpha$ -chloromethyl ketone (164 mg, 0.76 mmol, 1.2 eq; prepared from 4-tert-butylcyclohexanecarboxylic acid), provided 160 mg of the title compound as a white foam. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

$R_f = 0.45$  (50% EtOAc in  $\text{CH}_2\text{Cl}_2$ ).

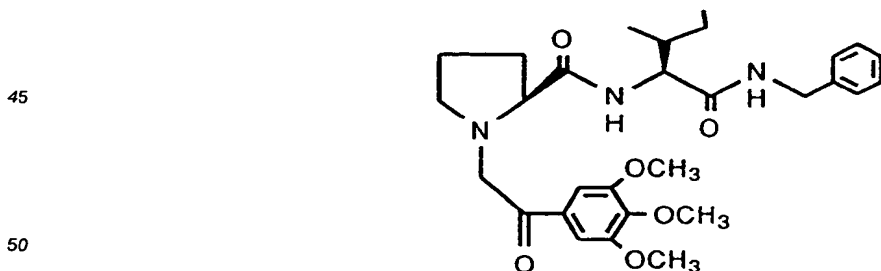
LSIMS = 498 (mass calculated for  $\text{C}_{30}\text{H}_{47}\text{N}_3\text{O}_3 = 497.73$ ).

**Example 89**

**L-Isoleucine, N-[1-(2-(Bicyclo[2.2.1]hept-2-yl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of L-proline-L-isoleucine benzylamide (100 mg, 0.32 mmol) with 2-norbornyl  $\alpha$ -chloromethyl ketone (82 mg, 0.48 mmol, 1.5 eq; prepared from 2-norbornanecarboxylic acid) provided 69 mg of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

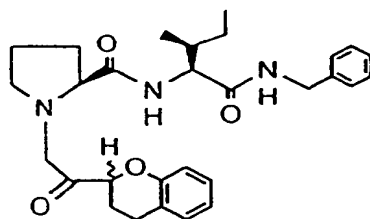
$R_f = 0.28$  (70% EtOAc in hexane).

LSIMS = 454 (mass calculated for  $\text{C}_{27}\text{H}_{39}\text{N}_3\text{O}_3 = 453.63$ ).

**Example 90**

**L-Isoleucine, N-[1-(2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of L-proline-L-isoleucine benzylamide (100 mg, 0.32 mmol) with 3,4,5-trimethoxyphenyl  $\alpha$ -chloromethyl ketone (116 mg, 0.47 mmol, 1.5 eq; prepared from 3,4,5-trimethoxybenzoic acid) provided 190 mg of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

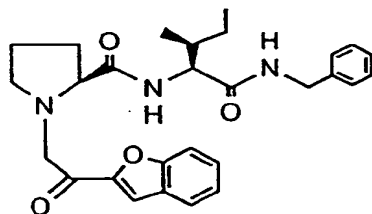
$R_f = 0.33$  (50% EtOAc in  $\text{CH}_2\text{Cl}_2$ ).

**Example 91**

**L-Isoleucine, N-[1-(2-(Chroman-2-yl)-2-Oxoethyl)-L-Prolyl] Benzylamide Hydrochloride Salt.** Using the procedure described in example 1e, treatment of L-proline-L-isoleucine benzylamide (200 mg, 0.63 mmol) with benzopyranyl  $\alpha$ -chloromethyl ketone (266 mg, 1.26 mmol, 2 eq; prepared from 3,4-dihydro-2H-1-benzopyran-2-carboxylic acid) provided the amine which was treated with HCl in ether to provide 200 mg of the hydrochloride salt of the title compound as a mixture of diastereomers. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

$R_f$  = 0.38 (for free base: 80% EtOAc in hexane).

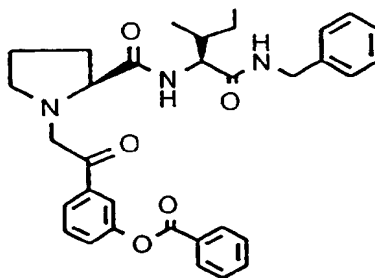
LSIMS  $[\text{M}-\text{HCl}] = 491$  (mass calculated for  $\text{C}_{29}\text{H}_{37}\text{N}_3\text{O}_4 + \text{HCl} = 528.09$ ).

**Example 92**

**L-Isoleucine, N-[1-(2-(Benzofuran-2-yl)-2-Oxoethyl)-L-Prolyl] Benzylamide Hydrochloride Salt.** Using the procedure described in example 1e, treatment of L-proline-L-isoleucine benzylamide (200 mg, 0.63 mmol) with benzofuranyl  $\alpha$ -chloromethyl ketone (246 mg, 1.26 mmol, 2 eq; prepared from 2-benzofurancarboxylic acid) provided the amine which was treated with HCl in ether to provide 200 mg of the hydrochloride salt of the title compound as a solid. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

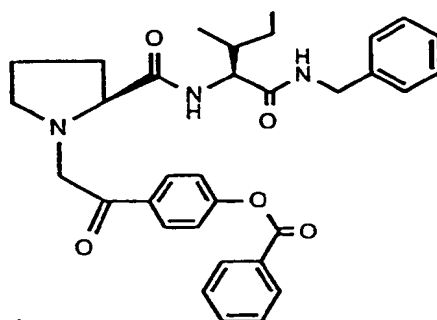
$R_f$  = 0.13 (for free base: 50% EtOAc in hexane).

LSIMS  $[\text{M}-\text{HCl}] = 476$  (mass calculated for  $\text{C}_{28}\text{H}_{33}\text{N}_3\text{O}_4 + \text{HCl} = 512.05$ ).

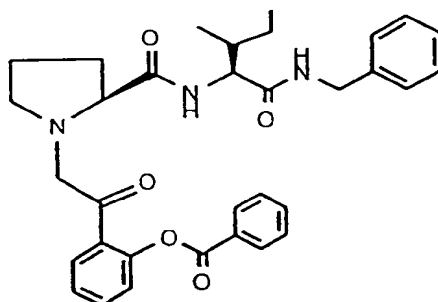
**Example 93**

**L-Isoleucin, N-[1-(2-(3-Benzoyloxyphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of L-proline-L-isoleucine benzylamide (200 mg, 0.63 mmol) with 3'-benzoyloxy-2-bromoacetophenone (302 mg, 0.95 mmol, 1.5 eq) provided 57 mg of the title compound as a solid. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

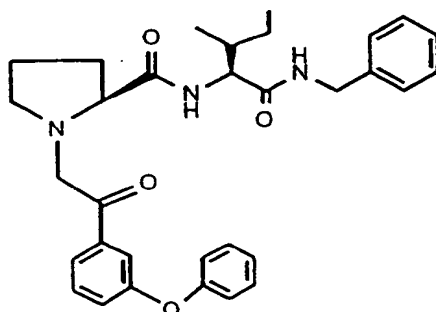
LSIMS = 556 (mass calculated for  $\text{C}_{33}\text{H}_{37}\text{N}_3\text{O}_5 = 555.68$ ).

**Example 94**

**L-Isoleucine, N-[1-(2-(4-Benzoyloxyphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of L-proline-L-isoleucine benzylamide (200 mg, 0.63 mmol) with 4'-benzoyloxy-2-bromoacetophenone (302 mg, 0.95 mmol, 1.5 eq) provided 171 mg of the title compound as a foam. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure. LSIMS = 556 (mass calculated for C<sub>33</sub>H<sub>37</sub>N<sub>3</sub>O<sub>5</sub> = 555.68).

**Example 95**

**L-Isoleucine, N-[1-(2-(2-Benzoyloxyphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of L-proline-L-isoleucine benzylamide (200 mg, 0.63 mmol) with 2'-benzoyloxy-2-bromoacetophenone (302 mg, 0.95 mmol, 1.5 eq) provided 120 mg of the title compound as a foam. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure. LSIMS = 556 (mass calculated for C<sub>33</sub>H<sub>37</sub>N<sub>3</sub>O<sub>5</sub> = 555.68).

**Example 96**

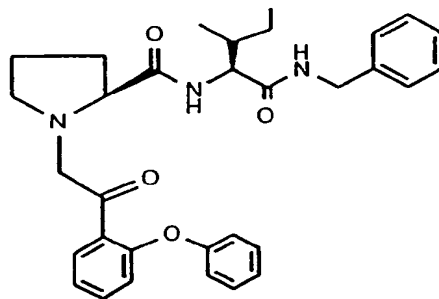
**L-Isoleucine, N-[1-(2-(3-Phenoxyphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of L-proline-L-isoleucine benzylamide (200 mg, 0.63 mmol) with 3'-phenoxy-2-chloroacetophenone (233 mg, 0.95 mmol, 1.5 eq; prepared from 3-phenoxybenzoic acid) provided 80 mg of the title compound as a foam. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was

consistent with the structure.

$R_f = 0.26$  (70% EtOAc in hexane).

LSIMS = 528 (mass calculated for  $C_{32}H_{37}N_3O_4 = 527.67$ ).

#### Example 97

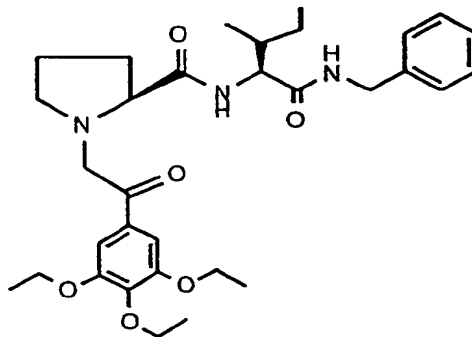


**L-Isoleucine, N-[1-(2-(2-Phenoxyphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of L-proline-L-isoleucine benzylamide (200 mg, 0.63 mmol) with 2'-phenoxy-2-chloroacetophenone (233 mg, 0.95 mmol, 1.5 eq; prepared from 2-phenoxybenzoic acid) provided 40 mg of the title compound as a foam. The 300 MHz,  $^1H$  NMR analysis of this compound was consistent with the structure.

$R_f = 0.31$  (70% EtOAc in hexane).

LSIMS = 528 (mass calculated for  $C_{32}H_{37}N_3O_4 = 527.67$ ).

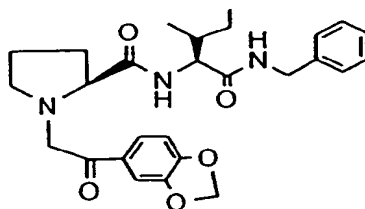
#### Example 98



**L-Isoleucine, N-[1-(2-(3,4,5-Triethoxyphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of L-proline-L-isoleucine benzylamide (200 mg, 0.63 mmol) with 3,4,5-triethoxyphenyl  $\alpha$ -chloromethyl ketone (271 mg, 0.95 mmol, 1.5 eq; prepared from 3,4,5-triethoxybenzoic acid), provided 45 mg of the title compound as a foam. The 300 MHz,  $^1H$  NMR analysis of this compound was consistent with the structure.

$R_f = 0.19$  (70% EtOAc in hexane).

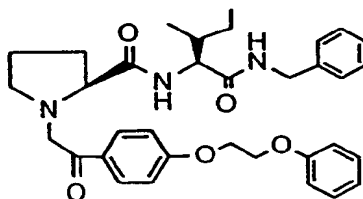
LSIMS = 568 (mass calculated for  $C_{32}H_{45}N_3O_6 = 567.73$ ).

**Example 99**

**L-Isoleucine, N-[1-(2-(Benzo[1,3]dioxol-5-yl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of L-proline-L-isoleucine benzylamide (200 mg, 0.63 mmol) with piperonyl  $\alpha$ -chloromethyl ketone (188 mg, 0.95 mmol, 1.5 eq; prepared from piperonylic acid), provided 84 mg of the title compound as a foam. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

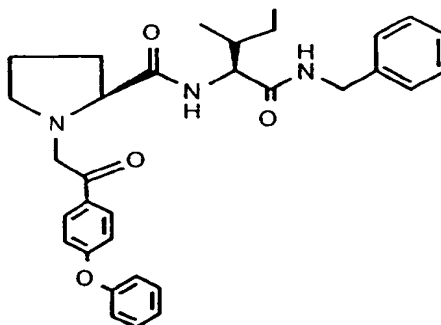
$R_f = 0.24$  (70% EtOAc in hexane).

LSIMS = 480 (mass calculated for  $\text{C}_{27}\text{H}_{33}\text{N}_3\text{O}_5 = 479.58$ ).

**Example 100**

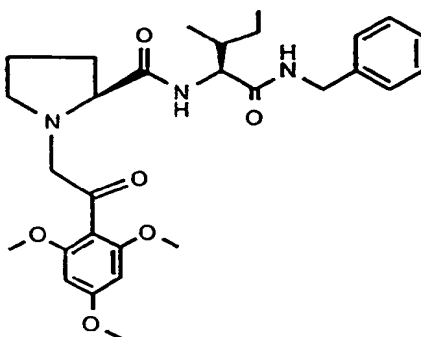
**L-Isoleucine, N-[1-{2-Oxo-2-[4-(2-Phenoxyethoxy)-Phenyl]-Ethyl}-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of L-proline-L-isoleucine benzylamide (200 mg, 0.63 mmol) with 4'-phenoxyethoxy-2-chloroacetophenone, (275 mg, 0.95 mmol, 1.5 eq; prepared from 4-(2-phenoxyethoxy)benzoic acid), provided 80 mg of the title compound as a crystalline solid. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

LSIMS = 572 (mass calculated for  $\text{C}_{34}\text{H}_{41}\text{N}_3\text{O}_5 = 571.72$ ).

**Example 101**

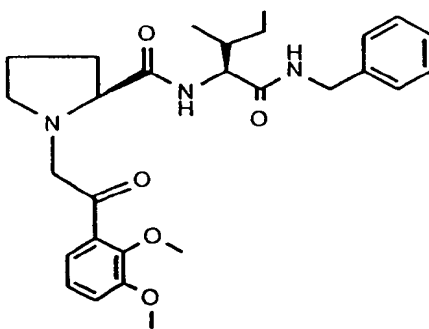
**L-Isoleucine, N-[1-(2-(4-Phenoxyphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of L-proline-L-isoleucine benzylamide (200 mg, 0.63 mmol) with 4'-phenoxy-2-bromoacetophenone (275 mg, 0.95 mmol, 1.5 eq) provided 142 mg of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

LSIMS = 528 (mass calculated for  $\text{C}_{32}\text{H}_{37}\text{N}_3\text{O}_4 = 527.67$ ).

**Example 102**

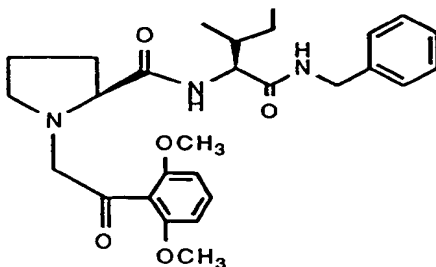
**L-Isoleucine, N-[1-(2-(2,4,6-Trimethoxyphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of L-proline-L-isoleucine benzylamide (200 mg, 0.63 mmol) with 2',4',6'-trimethoxyphenyl  $\alpha$ -bromomethyl ketone (273 mg, 0.95 mmol, 1.5 eq) provided 88 mg of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

LSIMS = 526 (mass calculated for  $\text{C}_{29}\text{H}_{39}\text{N}_3\text{O}_6$  = 525.65).

**Example 103**

**L-Isoleucine, N-[1-(2-(2,3-Dimethoxyphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of L-proline-L-isoleucine benzylamide (200 mg, 0.63 mmol) with 2',3'-dimethoxyphenyl  $\alpha$ -chloromethyl ketone (190 mg, 0.88 mmol, 1.4 eq; prepared from 2,3-dimethoxybenzoic acid) provided 34 mg of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

LSIMS = 496 (mass calculated for  $\text{C}_{28}\text{H}_{37}\text{N}_3\text{O}_5$  = 495.62).

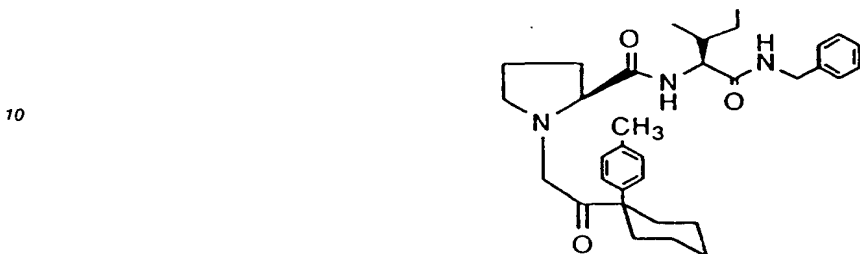
**Example 104**

**L-Isoleucine, N-[1-(2-(2,6-Dimethoxyphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of L-proline-L-isoleucine benzylamide (200 mg, 0.63 mmol) with 2',6'-



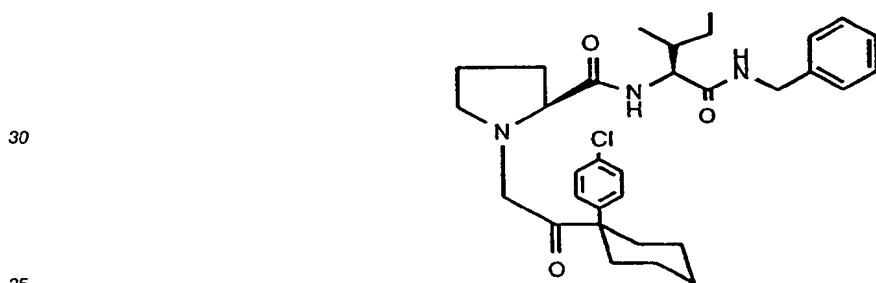
dimethoxyphenyl  $\alpha$ -bromomethyl ketone (326 mg, 1.26 mmol, 2.0 eq) provided 80 mg of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.  
LSIMS = 496 (mass calculated for  $\text{C}_{28}\text{H}_{37}\text{N}_3\text{O}_5$  = 495.62).

### 5 Example 105



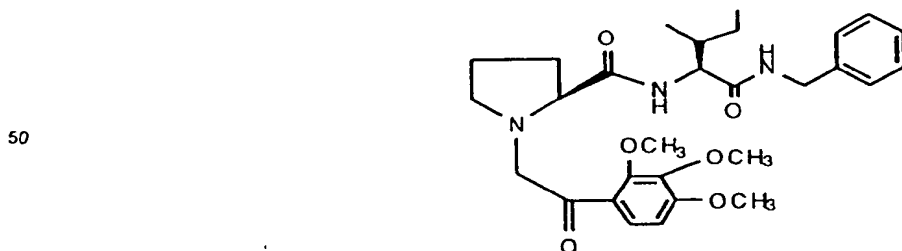
**L-Isoleucine, N-[1-(2-(1-(4-Methylphenyl)cyclohexyl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of L-proline-L-isoleucine benzylamide (200 mg, 0.63 mmol) with 1-(4-methylphenyl)cyclohexyl  $\alpha$ -chloromethyl ketone (237 mg, 1.26 mmol, 2.0 eq; prepared from 1-(4-methylphenyl)-1-cyclohexanecarboxylic acid) provided 30 mg of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.  
LSIMS = 532 (mass calculated for  $\text{C}_{33}\text{H}_{45}\text{N}_3\text{O}_3$  = 531.74).

### 25 Example 106



**L-Isoleucine, N-[1-(2-(1-(4-Chlorophenyl)cyclohexyl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of L-proline-L-isoleucine benzylamide (200 mg, 0.63 mmol) with 1-(4-chlorophenyl)cyclohexyl  $\alpha$ -chloromethyl ketone (342 mg, 1.26 mmol, 2.0 eq; prepared from 1-(4-chlorophenyl)-1-cyclohexanecarboxylic acid) provided 30 mg of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.  
LSIMS = 552 (mass calculated for  $\text{C}_{32}\text{H}_{42}\text{ClN}_3\text{O}_3$  = 552.16).

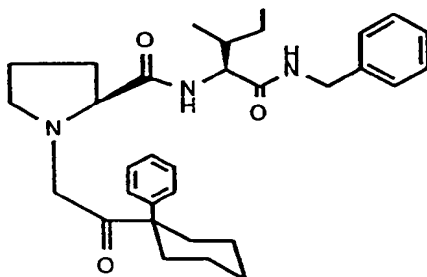
### 45 Example 107



**L-Isoleucine, N-[1-(2-(2,3,4-Trimethoxyphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of L-proline-L-isoleucine benzylamide (200 mg, 0.63 mmol) with 2,3,4-trimethoxyphenyl  $\alpha$ -bromomethyl ketone (273 mg, 0.95 mmol, 1.5 eq) provided 100 mg of the title

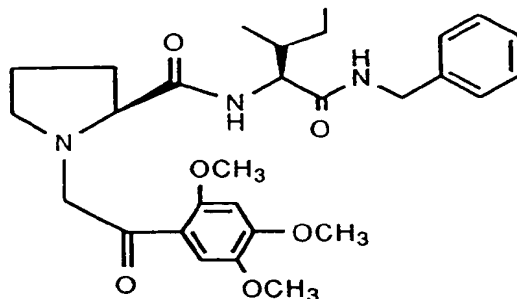
compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.  
LSIMS = 526 (mass calculated for  $\text{C}_{29}\text{H}_{39}\text{N}_3\text{O}_6$  = 525.65).

#### Example 108



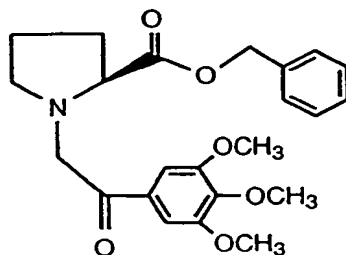
**L-Isoleucine, N-[1-(2-(1-Phenylcyclohexyl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of L-proline-L-isoleucine benzylamide (200 mg, 0.63 mmol) with 1-phenylcyclohexyl  $\alpha$ -chloromethyl ketone (224 mg, 0.95 mmol, 1.5 eq; prepared from 1-phenyl-1-cyclohexanecarboxylic acid) provided 40 mg of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.  
LSIMS = 518 (mass calculated for  $\text{C}_{32}\text{H}_{43}\text{N}_3\text{O}_3$  = 517.72).

#### Example 109



**L-Isoleucine, N-[1-(2-(2,4,5-Trimethoxyphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide.** Using the procedure described in example 1e, treatment of L-proline-L-isoleucine benzylamide (200 mg, 0.63 mmol) with 2',4',5'-trimethoxyphenyl  $\alpha$ -bromomethyl ketone (274 mg, 0.95 mmol, 1.5 eq) provided 40 mg of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.  
LSIMS = 526 (mass calculated for  $\text{C}_{29}\text{H}_{39}\text{N}_3\text{O}_6$  = 525.65).

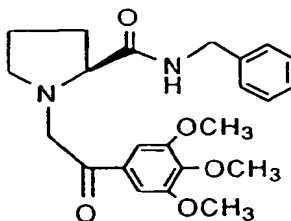
#### Example 110



**1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] L-Proline Benzyl Ester Hydrochloride.** Using the procedure described in example 1e, treatment of L-proline benzyl ester hydrochloride (1.00 g, 4.14 mmol) and 3,4,5-trimethoxyphenyl  $\alpha$ -bromomethyl ketone (2.4 g, 8.27 mmol) provided the amine ( $R_f$  = 0.37: 50% EtOAc in

hexane). The amine intermediate was treated with HCl in ether and dried in vacuo to provide 1.58 g of the title compound as a solid. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

### 5 Example 111



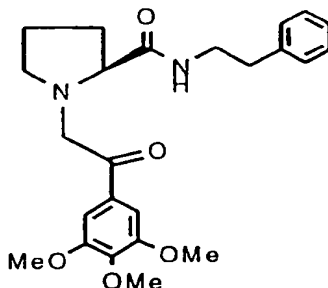
#### L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] Benzylamide Hydrochloride.

a) 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] L-Proline Hydrochloride. The hydrochloride salt of 1-[2-(3,4,5-trimethoxyphenyl)-2-oxoethyl] L-proline benzyl ester (1.53 g), was dissolved in ethanol (100 mL) and treated with 10% palladium on carbon (150 mg). The flask was purged with argon, purged with hydrogen and left to stir under hydrogen atmosphere (1 atm) until the reaction appeared complete by TLC. The catalyst was removed by filtration through Celite and solvent removed in vacuo to provide 1.15 g of the title compound as a solid. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

b) L-Proline 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] Benzylamide Hydrochloride. To an oven-dried round bottomed flask was added N-[2-(3,4,5-trimethoxyphenyl)-2-oxoethyl]-L-proline hydrochloride, (300 mg, 0.83 mmol, 1.0 eq), benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (369 mg, 0.83 mmol, 1.0 eq.) and anhydrous tetrahydrofuran (10 mL). The slurry was cooled to 0 °C and treated with triethylamine (3.0 eq). After stirring 10 minutes at 0 °C, benzylamine (0.27 mL, 2.5 mmol, 3.0 eq) was added and the reaction mixture was allowed to warm to 22 °C over a one hour period. The solvent was removed in vacuo and the resulting residue was taken up in EtOAc (100 mL). The organic layer was washed with 5% citric acid (100 mL), sat.  $\text{NaHCO}_3$  (100 mL), sat. aq.  $\text{NaCl}$  (100 mL), dried ( $\text{MgSO}_4$ ) and concentrated in vacuo. The residue was purified by flash chromatography and treated with HCl in ether to provide 128 mg of the title compound as a powder. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

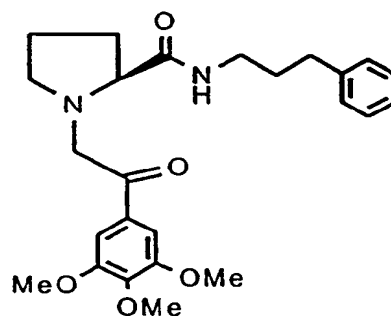
LSIMS  $[\text{M}-\text{HCl}] = 413$  (mass calculated for  $\text{C}_{23}\text{H}_{28}\text{N}_2\text{O}_5 + \text{HCl} = 448.93$ ).

### 40 Example 112



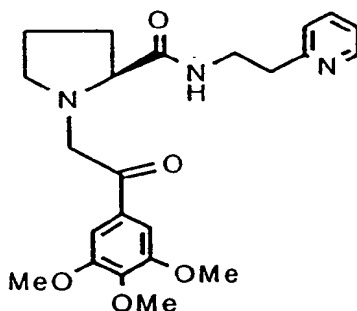
L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 2-Phenethylamide Hydrochloride. Following the procedure described in Example 111b, the coupling of N-[2-(3,4,5-trimethoxyphenyl)-2-oxoethyl]-L-proline hydrochloride (300 mg, 0.83 mmol) and phenethylamine (0.31 mL, 2.5 mmol) provided, after treatment with HCl in  $\text{Et}_2\text{O}$ , 199 mg of the title compound as a powder. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

LSIMS  $[\text{M}-\text{HCl}] = 427$  (mass calculated for  $\text{C}_{24}\text{H}_{30}\text{N}_2\text{O}_5 + \text{HCl} = 462.98$ ).

**Example 113**

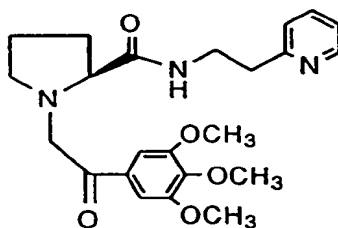
**L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 3-Phenylpropylamide Hydrochloride.** Following the procedure described in Example 111b, the coupling of N-[2-(3,4,5-trimethoxyphenyl)-2-oxoethyl]-L-proline hydrochloride (300 mg, 0.83 mmol) and 3-phenylpropylamine (0.36 mL, 2.5 mmol) provided, after treatment with HCl in Et<sub>2</sub>O, 120 mg of the title compound as a powder. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure.

LSIMS [M-HCl] = 441 (mass calculated for C<sub>25</sub>H<sub>32</sub>N<sub>2</sub>O<sub>5</sub> + HCl = 477.00).

**Example 114**

**L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 4-Pyridylbutylamide Hydrochloride.** Following the procedure described in Example 111b, the coupling of N-[2-(3,4,5-trimethoxyphenyl)-2-oxoethyl]-L-proline hydrochloride (300 mg, 0.83 mmol) and 4-pyridylbutylamine (0.39 mL, 2.5 mmol) provided, after treatment with HCl in Et<sub>2</sub>O, 170 mg of the title compound as a powder. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure.

LSIMS [M-HCl] = 455 (mass calculated for C<sub>26</sub>H<sub>34</sub>N<sub>2</sub>O<sub>5</sub> + HCl = 491.03).

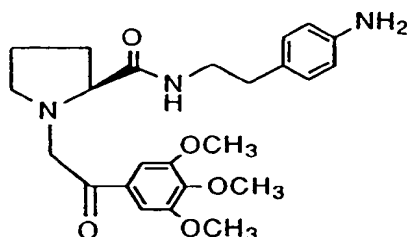
**Example 115**

**L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 2-(Pyrid-2-yl)ethylamide Dihydrochloride.** Following the procedure described in Example 111b, the coupling of N-[2-(3,4,5-trimethoxyphenyl)-2-oxoethyl]-L-proline hydrochloride (300 mg, 0.83 mmol) and 2-(2-aminoethyl)pyridine (0.4 mL, 2.5 mmol) provided, after treatment with HCl in Et<sub>2</sub>O, 193 mg of the title compound as a powder. The 300 MHz, <sup>1</sup>H NMR analysis of

this compound was consistent with the structure.

LSIMS [M-2HCl] = 429 (mass calculated for  $C_{23}H_{29}N_3O_5 \times 2HCl = 500.43$ ).

#### Example 116



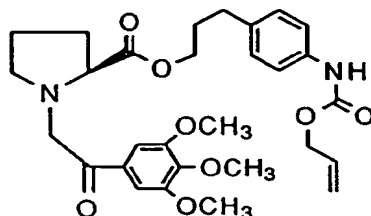
#### L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 2-(4-aminophenyl)ethylamide Dihydrochloride.

Following the procedure described in Example 111b, the coupling of N-[2-(3,4,5-trimethoxyphenyl)-2-oxoethyl]-L-proline hydrochloride (300 mg, 0.83 mmol) and (4-aminophenyl)ethylamine (0.4 mL, 2.5 mmol) provided, after treatment with HCl in  $Et_2O$ , 102 mg of the title compound as a powder. The 300 MHz,  $^1H$  NMR analysis of this compound was consistent with the structure.

$R_f = 0.27$  (for free base: EtOAc)

LSIMS [M-2HCl] = 442 (mass calculated for  $C_{24}H_{31}N_3O_5 \times 2HCl = 514.45$ ).

#### Example 117



#### L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 3-(4-[N-Carboallyloxy]aminophenyl)propyl Ester Hydrochloride.

a) 3-(4-Aminophenyl)propanol. To a round bottomed flask equipped with a magnetic stirrer was added 4-nitrocinnamyl alcohol (2.0 g, 11.16 mmol), 10% Pd on carbon (200 mg) and absolute ethanol (150 mL). The solution was purged with hydrogen and stirred at 22 °C under a hydrogen atmosphere. When TLC indicated the reaction was complete (4 h), the solution was purged with argon and filtered through Celite. The filtrate was concentrated *in vacuo* to provide 3-(4-aminophenyl)propanol, 1.72 g (>100%), as a viscous oil which solidified on standing.  $R_f = 0.17$  (50% EtOAc in hexane).

b) 3-(4-(N-Carboallyloxy)-aminophenyl)propanol. To a round bottomed flask was added the 3-(4-aminophenyl)propanol (1.3 g, 8.6 mmol), pyridine (1.0 mL, 12 mmol) and dichloromethane (25 mL). The solution was cooled to 0 °C and treated with allyl chloroformate (1.0 mL, 9.4 mmol). After allowing to warm to 22 °C over 1 hour, the reaction mixture was diluted with dichloromethane and washed twice with 1N HCl, followed by sat.  $NaHCO_3$ , water and sat. aq. NaCl. The organic extract was dried ( $MgSO_4$ ) and concentrated *in vacuo*. Purification by flash chromatography (50% EtOAc in hexane), provided 1.77 g (88%) of the title compound as a clear oil which solidified on standing. The  $^1H$  NMR analysis of this compound was consistent with the structure.

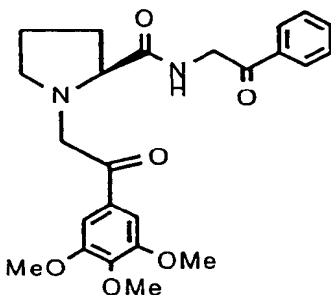
$R_f = 0.37$  (60% EtOAc in hexane).

c) L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 3-(4-[N-Carboallyloxy]aminophenyl)propyl Ester Hydrochloride. Following the procedure described in Example 111b, the coupling of N-[2-(3,4,5-trimethoxyphenyl)-2-oxoethyl]-L-proline hydrochloride (300 mg, 0.83 mmol) and 3-(4-(N-carboallyloxy)-aminophenyl)propanol (218 mg, 0.83 mmol) provided, after treatment with HCl in  $Et_2O$ , 65 mg of the title compound as a foam. The 300 MHz,  $^1H$  NMR analysis of this compound was consistent with the

structure.

LSIMS [M-HCl] = 542 (mass calculated for  $C_{29}H_{36}N_2O_8 + HCl = 577.08$ ).

### Example 118

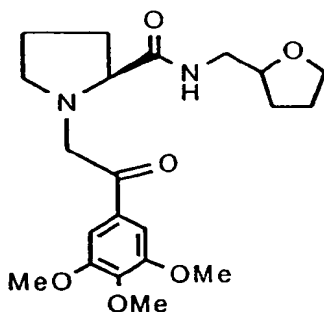


**L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 2-Phenyl-2-oxoethylamide.** Following the procedure described in Example 111b, the coupling of N-[2-(3,4,5-trimethoxyphenyl)-2-oxoethyl]-L-proline hydrochloride (200 mg, 0.56 mmol) and 2-aminoacetophenone hydrochloride (286 mg, 1.67 mmol) provided 54 mg of the title compound as a powder. The 300 MHz,  $^1H$  NMR analysis of this compound was consistent with the structure.

Rf = 0.46 (EtOAc)

LSIMS = 442 (mass calculated for  $C_{24}H_{28}N_2O_6 = 440.50$ ).

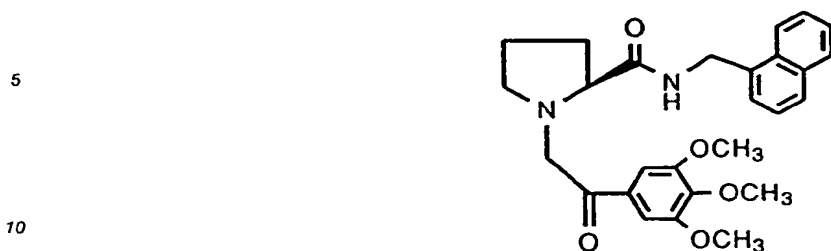
### Example 119



**L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] Tetrahydrofurfurylamide.** Following the procedure described in Example 111b, the coupling of N-[2-(3,4,5-trimethoxyphenyl)-2-oxoethyl]-L-proline hydrochloride (200 mg, 0.56 mmol) and tetrahydrofurfurylamine (0.17 mL, 1.67 mmol) provided 104 mg of the title compound as a powder. The 300 MHz,  $^1H$  NMR analysis of this compound was consistent with the structure.

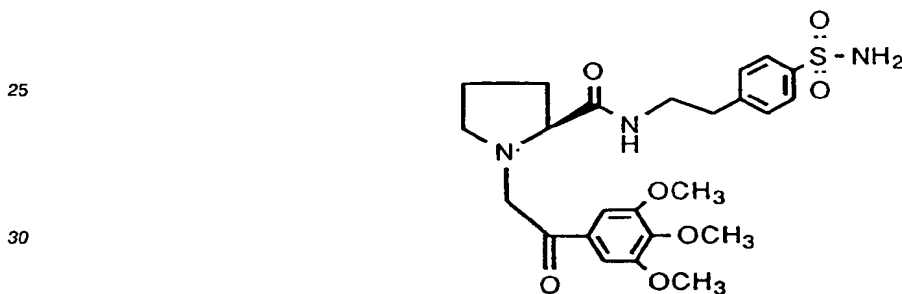
Rf = 0.20 (EtOAc)

LSIMS = 407 (mass calculated for  $C_{21}H_{30}N_2O_6 = 406.48$ ).

**Example 120**

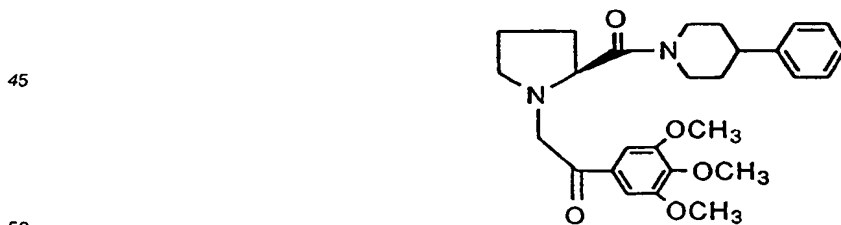
**L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] Naphthalen-1-ylmethylamide.** Following the procedure described in Example 111b, the coupling of N-[2-(3,4,5-trimethoxyphenyl)-2-oxoethyl]-L-proline hydrochloride (300 mg, 0.83 mmol) and 1-naphthyl methylamine (0.37 mL, 2.5 mmol) provided 150 mg of the title compound as a powder. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure.

LSIMS = 463 (mass calculated for C<sub>27</sub>H<sub>30</sub>N<sub>2</sub>O<sub>5</sub> = 462.55).

**Example 121**

**L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 2-(4-Sulfamoylphenyl)ethylamide.** Following the procedure described in Example 111b, the coupling of N-[2-(3,4,5-trimethoxyphenyl)-2-oxoethyl]-L-proline hydrochloride (300 mg, 0.83 mmol) and 4-(2-aminoethyl)benzene sulfonamide (334 mg, 1.67 mmol) provided 300 mg of the title compound as a powder. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure.

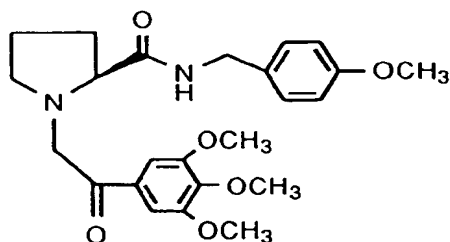
LSIMS = 506 (mass calculated for C<sub>24</sub>H<sub>31</sub>N<sub>3</sub>SO<sub>7</sub> = 505.60).

**Example 122**

**L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 4-Phenylpiperid nylamide.** Following the procedure described in Example 111b, the coupling of N-[2-(3,4,5-trimethoxyphenyl)-2-oxoethyl]-L-proline hydrochloride (250 mg, 0.69 mmol) and 4-phenylpiperidine (336 mg, 2.1 mmol) provided 67 mg of the title compound as a foam. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure.

R<sub>f</sub> = 0.22 (EtOAc)

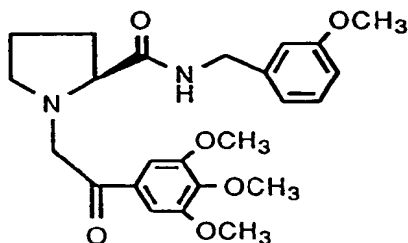
LSIMS = 467 (mass calculated for C<sub>27</sub>H<sub>34</sub>N<sub>2</sub>O<sub>5</sub> = 466.58).

**Example 123**

**L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 4-Methoxybenzamide Hydrochloride.** Following the procedure described in Example 111b, the coupling of N-[2-(3,4,5-trimethoxyphenyl)-2-oxoethyl]-L-proline hydrochloride (250 mg, 0.69 mmol) and 4-methoxybenzylamine (0.27 mL, 2.1 mmol) provided, after treatment with HCl in Et<sub>2</sub>O, 90 mg of the title compound as a powder. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure.

R<sub>f</sub> = 0.38 (free base in EtOAc)

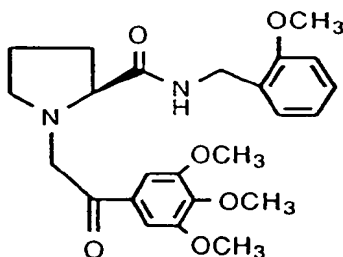
LSIMS [M-HCl] = 443 (mass calculated for C<sub>24</sub>H<sub>30</sub>N<sub>2</sub>O<sub>6</sub> + HCl = 478.98).

**Example 124**

**L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 3-Methoxybenzamide Hydrochloride.** Following the procedure described in Example 111b, the coupling of N-[2-(3,4,5-trimethoxyphenyl)-2-oxoethyl]-L-proline hydrochloride (250 mg, 0.69 mmol) and 3-methoxybenzylamine (0.27 mL, 2.1 mmol) provided, after treatment with HCl in Et<sub>2</sub>O, 90 mg of the title compound as a powder. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure.

R<sub>f</sub> = 0.38 (free base in EtOAc)

LSIMS [M-HCl] = 443 (mass calculated for C<sub>24</sub>H<sub>30</sub>N<sub>2</sub>O<sub>6</sub> + HCl = 478.98).

**Example 125**

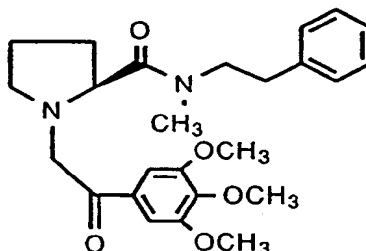
**L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 2-Methoxybenzamide Hydrochloride.** Following the procedure described in Example 111b, the coupling of N-[2-(3,4,5-trimethoxyphenyl)-2-oxoethyl]-L-proline hydrochloride (250 mg, 0.69 mmol) and 2-methoxybenzylamine (0.27 mL, 2.1 mmol) provided, after treatment with HCl in Et<sub>2</sub>O, 120 mg of the title compound as a powder. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure.



$R_f = 0.38$  (free base in EtOAc)

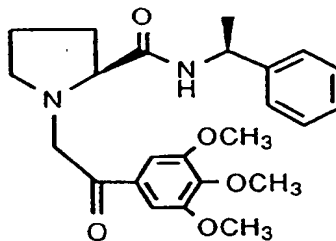
LSIMS [M-HCl] = 443 (mass calculated for  $C_{24}H_{30}N_2O_6 + HCl = 478.98$ ).

### Example 126



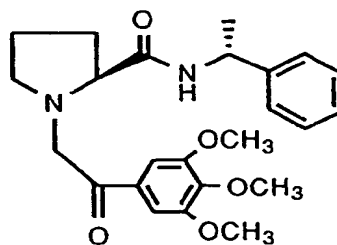
**L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] N-Methylphenethylamide Hydrochloride.** Following the procedure described in Example 111b, the coupling of N-[2-(3,4,5-trimethoxyphenyl)-2-oxoethyl]-L-proline hydrochloride (250 mg, 0.69 mmol) and N-methyl phenethylamine (0.30 mL, 2.1 mmol) provided, after treatment with HCl in Et<sub>2</sub>O, 20 mg of the title compound as a powder. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure. LSIMS [M-HCl] = 441 (mass calculated for  $C_{25}H_{32}N_2O_5 + HCl = 477.00$ ).

### Example 127



**L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] (S)-α-methylbenzylamide Hydrochloride.** Following the procedure described in Example 111b, the coupling of N-[2-(3,4,5-trimethoxyphenyl)-2-oxoethyl]-L-proline hydrochloride (250 mg, 0.69 mmol) and (S)-(-)-α-methylbenzylamine (0.27 mL, 2.1 mmol) provided, after treatment with HCl in Et<sub>2</sub>O, 160 mg of the title compound as a powder. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure. LSIMS [M-HCl] = 427 (mass calculated for  $C_{24}H_{30}N_2O_5 + HCl = 462.98$ ).

### Example 128

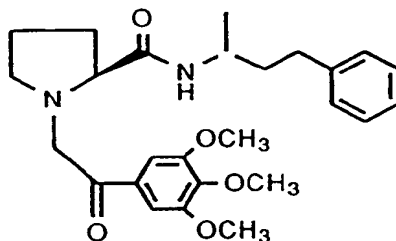


**L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] (R)-α-methylbenzylamid Hydrochloride.** Following the procedure described in Example 111b, the coupling of N-[2-(3,4,5-trimethoxyphenyl)-2-oxoethyl]-L-proline hydrochloride (250 mg, 0.69 mmol) and (R)-(+)-α-methylbenzylamine (0.27 mL, 2.1 mmol) provided, after treatment with HCl in Et<sub>2</sub>O, 190 mg of the title compound as a powder. The 300 MHz, <sup>1</sup>H NMR

analysis of this compound was consistent with the structure.

LSIMS [M-HCl] = 427 (mass calculated for  $C_{24}H_{30}N_2O_5 + HCl = 462.98$ ).

#### Example 129

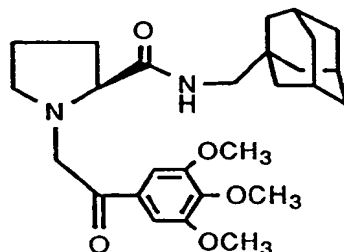


#### **L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 1-methyl-3-phenylpropylamide Hydrochloride.**

Following the procedure described in Example 111b, the coupling of N-[2-(3,4,5-trimethoxyphenyl)-2-oxoethyl]-L-proline hydrochloride (250 mg, 0.69 mmol) and 1-methyl-3-phenylpropylamine (0.34 mL, 2.1 mmol) provided, after treatment with HCl in  $Et_2O$ , 40 mg of the title compound as a foam. The 300 MHz,  $^1H$  NMR analysis of this compound was consistent with the structure.

LSIMS [M-HCl] = 455 (mass calculated for  $C_{26}H_{34}N_2O_5 + HCl = 491.03$ ).

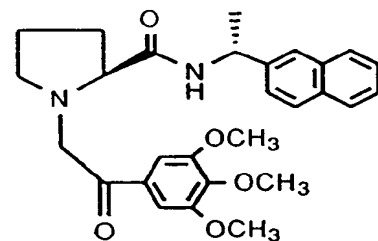
#### Example 130



**L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] Adamant-1-ylmethylamide Hydrochloride.** Following the procedure described in Example 111b, the coupling of N-[2-(3,4,5-trimethoxyphenyl)-2-oxoethyl]-L-proline hydrochloride (250 mg, 0.69 mmol) and 1-adamantylmethylamine (0.37 mL, 2.1 mmol) provided, after treatment with HCl in  $Et_2O$ , 100 mg of the title compound as a powder. The 300 MHz,  $^1H$  NMR analysis of this compound was consistent with the structure.

LSIMS [M-HCl] = 471 (mass calculated for  $C_{27}H_{38}N_2O_5 + HCl = 507.07$ ).

#### Example 131



#### **L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 1-(R)-(1-naphthyl)ethylamide Hydrochloride.**

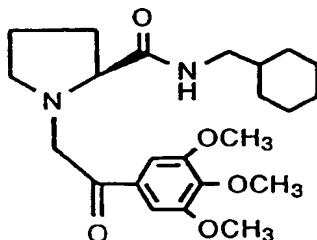
Following the procedure described in Example 111b, the coupling of N-[2-(3,4,5-trimethoxyphenyl)-2-oxoethyl]-L-proline hydrochloride (250 mg, 0.69 mmol) and (R)-1-(1-naphthyl)ethylamine (0.34 mL, 2.1 mmol) provided, after treatment with HCl in  $Et_2O$ , 137 mg of the title compound as a powder. The 300 MHz,  $^1H$  NMR analysis of this compound was consistent with the structure.

LSIMS [M-HCl] = 477 (mass calculated for  $C_{28}H_{32}N_2O_5 + HCl = 513.04$ ).

### Example 132

5

10



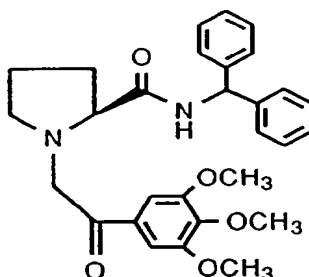
15 **L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] Cyclohexylmethylamide.** Following the procedure described in Example 111b, the coupling of N-[2-(3,4,5-trimethoxyphenyl)-2-oxoethyl]-L-proline hydrochloride (250 mg, 0.69 mmol) and cyclohexylmethylamine (0.27 mL, 2.1 mmol), provided 138 mg of the title compound as a powder. The 300 MHz,  $^1H$  NMR analysis of this compound was consistent with the structure.

20 LSIMS = 419 (mass calculated for  $C_{24}H_{34}N_2O_5 = 418.54$ ).

### Example 133

25

30



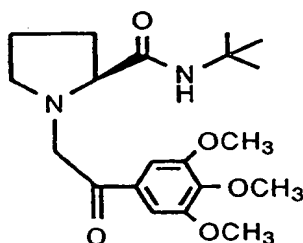
35 **L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] Diphenylmethylamide Hydrochloride.** Following the procedure described in Example 111b, the coupling of N-[2-(3,4,5-trimethoxyphenyl)-2-oxoethyl]-L-proline hydrochloride (250 mg, 0.69 mmol) and aminodiphenylmethane (0.12 mL, 0.69 mmol) provided, after treatment with HCl in  $Et_2O$ , 132 mg of the title compound as a powder. The 300 MHz,  $^1H$  NMR analysis of this compound was consistent with the structure.

40 LSIMS [M-HCl] = 489 (mass calculated for  $C_{29}H_{32}N_2O_5 + HCl = 525.05$ ).

### Example 134

45

50

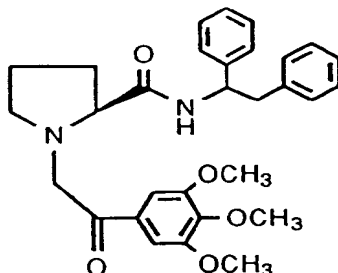


55 **L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] tert-Butylamide Hydrochloride.** Following the procedure described in Example 111b, the coupling of N-[2-(3,4,5-trimethoxyphenyl)-2-oxoethyl]-L-proline hydrochloride (250 mg, 0.69 mmol) and t-butylamine (0.22 mL, 2.1 mmol) provided, after treatment with HCl in  $Et_2O$ , 146 mg of the title compound as a powder. The 300 MHz,  $^1H$  NMR analysis of this compound was consistent with the structure.

R<sub>f</sub> = 0.42 (for free base: EtOAc)

LSIMS [M-HCl] = 379 (mass calculated for C<sub>20</sub>H<sub>30</sub>N<sub>2</sub>O<sub>5</sub> + HCl = 414.93).

### Example 135

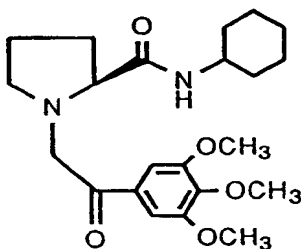


**L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 1,2-Diphenylethylamide Hydrochloride.** Following the procedure described in Example 111b, the coupling of N-[2-(3,4,5-trimethoxyphenyl)-2-oxoethyl]-L-proline hydrochloride (250 mg, 0.69 mmol) and 1,2-diphenylethylamine (0.40 mL, 2.1 mmol) provided, after treatment with HCl in Et<sub>2</sub>O, 95 mg of the title compound as a powder. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure.

R<sub>f</sub> = 0.56 (free base in EtOAc)

LSIMS [M-HCl] = 503 (mass calculated for C<sub>30</sub>H<sub>34</sub>N<sub>2</sub>O<sub>5</sub> + HCl = 539.07).

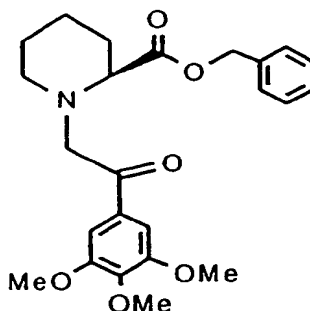
### Example 136



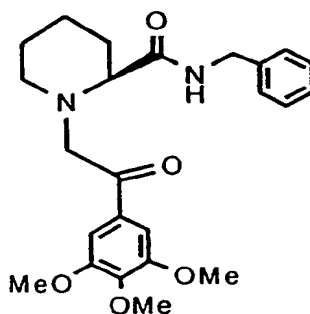
**L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] Cyclohexyl amide Hydrochloride.** Following the procedure described in Example 111b, the coupling of N-[2-(3,4,5-trimethoxyphenyl)-2-oxoethyl]-L-proline hydrochloride (250 mg, 0.69 mmol) and cyclohexylamine (0.24 mL, 2.1 mmol) provided, after treatment with HCl in Et<sub>2</sub>O, 147 mg of the title compound as a powder. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure.

R<sub>f</sub> = 0.34 (free base in EtOAc)

LSIMS [M-HCl] = 405 (mass calculated for C<sub>22</sub>H<sub>32</sub>N<sub>2</sub>O<sub>5</sub> + HCl = 440.97).

**Example 137**

15 **1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] L-Homoproline Benzyl Ester Hydrochloride.** Using the procedure described in example 1e, L-homoproline benzyl ester tosylate salt (5.0 g, 12.77 mmol) and 3,4,5-trimethoxyphenyl  $\alpha$ -bromomethyl ketone (7.4 g, 25.6 mmol) provided 5.4 g of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

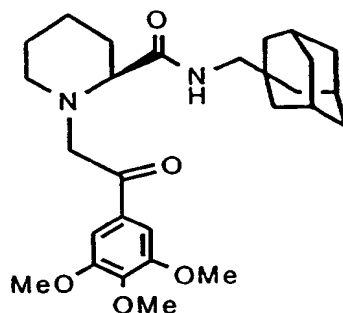
**Example 138**

35 **L-Homoproline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] Benzylamide Hydrochloride.**

a) **1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] L-Homoproline Benzyl Ester Hydrochloride.** 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] L-Homoproline Benzyl Ester Hydrochloride was reductively cleaved using the procedure described in Example 118 to provide 4.46 g of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

b) **L-Homoproline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] Benzylamide Hydrochloride.** Following the procedure described in Example 111b, the coupling of N-[2-(3,4,5-trimethoxyphenyl)-2-oxoethyl]-L-homoprolinehydrochloride (200 mg, 0.53 mmol) and benzylamine (0.09 mL, 0.80 mmol) provided, after treatment with HCl in Et<sub>2</sub>O, 112 mg of the title compound as a powder. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

LSIMS [M-HCl] = 427 (mass calculated for C<sub>24</sub>H<sub>30</sub>N<sub>2</sub>O<sub>5</sub> + HCl = 462.98).

**Example 139**

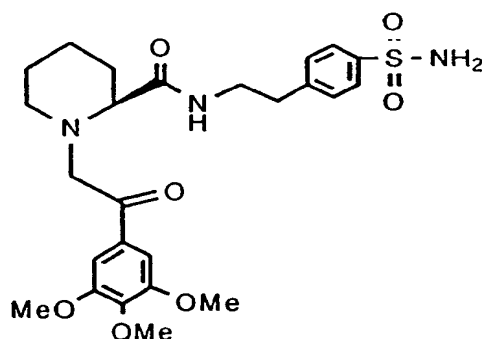
**L-Homoproline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] Adamant-1-ylmethylamide Hydrochloride.** Following the procedure described in Example 111b, the coupling of N-[2-(3,4,5-trimethoxyphenyl)-2-oxoethyl]-L-homoproline hydrochloride (200 mg, 0.53 mmol) and 1-adamantylmethylamine (0.19 mL, 1.07 mmol) provided, after treatment with HCl in Et<sub>2</sub>O, 59 mg of the title compound as a powder. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure.

LSIMS [M-HCl] = 485 (mass calculated for C<sub>28</sub>H<sub>40</sub>N<sub>2</sub>O<sub>5</sub> + HCl = 521.10).

**Example 140**

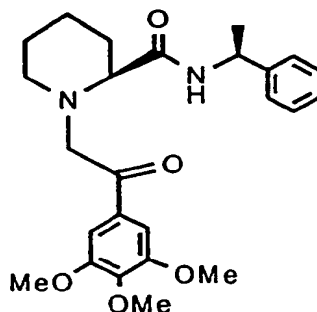
**L-Homoproline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] tetrahydrofurfurylamide.** Following the procedure described in Example 111b, the coupling of N-[2-(3,4,5-trimethoxyphenyl)-2-oxoethyl]-L-homoproline hydrochloride (200 mg, 0.53 mmol) and tetrahydrofurfurylamine (0.17 mL, 1.6 mmol), provided 100 mg of the title compound as a sticky solid. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure.

LSIMS = 421 (mass calculated for C<sub>22</sub>H<sub>32</sub>N<sub>2</sub>O<sub>6</sub> = 420.51).

**Example 141**

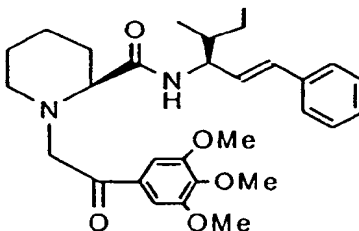
**L-Homoproline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 2-(4-Sulfamoylphenyl)ethylamide.** Following the procedure described in Example 111b, the coupling of N-[2-(3,4,5-trimethoxyphenyl)-2-oxoethyl]-L-homoproline hydrochloride (200 mg, 0.53 mmol) and 4-(2-aminoethyl)benzenesulfonamide (214 mg, 1.06 mmol) provided 25 mg of the title compound as a solid. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure.

LSIMS = 520 (mass calculated for C<sub>25</sub>H<sub>33</sub>N<sub>3</sub>O<sub>7</sub>S = 519.62).

**Example 142**

**L-Homoproline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] (S)-α-methylbenzylamide Hydrochloride.** Following the procedure described in Example 111b, the coupling of N-[2-(3,4,5-trimethoxyphenyl)-2-oxoethyl]-L-homoproline hydrochloride (200 mg, 0.53 mmol) and (S)-(-)-α-methylbenzylamine (0.21 mL, 1.6 mmol) provided, after treatment with HCl in Et<sub>2</sub>O, 107 mg of the title compound as a powder. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure.

LSIMS [M-HCl] = 441 (mass calculated for C<sub>25</sub>H<sub>32</sub>N<sub>2</sub>O<sub>5</sub> + HCl = 477.00).

**Example 143**

**L-Homoproline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] (1(S)-[2'-(S)-methylpropyl]-3-phenylprop-2-E-enyl)-amide.**

a) *trans* 1-Phenyl-3-(S)-[[[(1,1-dimethylethoxy)carbonyl]-amino]-4-(S)-methylhexa-1-ene]. Into a 1-L round bottomed flask equipped with a magnetic stirrer was added diethyl benzylphosphonate (14.3 mL, 15.8 g, 69.37 mmol, 1.2 eq.) and THF (500 mL). The flask was purged with argon and cooled to -78 °C. A 1 M solution of NaN(SiMe<sub>3</sub>)<sub>2</sub> in THF (74.1 mL, 74.1 mmol, 1.2 eq.) was added dropwise to the phosphonate, and the color changed from colorless to pale yellow. After stirring 30 min at -78 °C, a solution of Boc-L-isoleucinal (13.6 g, 63.1 mmol; prepared as described earlier: Saari, W. S.; Fisher, T. E. *Synthesis* **1990**, 453-454.) in THF (50 mL) was added dropwise. The reaction mixture was stirred at -78 °C for 30 min, then allowed to warm up to 0 °C over a 2 hour period. The solution was evaporated to dryness and the resulting colorless oil was dissolved in Et<sub>2</sub>O (250 mL). The ether solution was washed with sat. aq. NH<sub>4</sub>Cl (50 mL), sat. aq. NaCl (25 mL), dried (MgSO<sub>4</sub>) and evaporated to a residue. The residue was purified by flash chromatography (5% EtOAc in hexane) to provide 8.7 g (48%) of the title compound as a colorless oil.

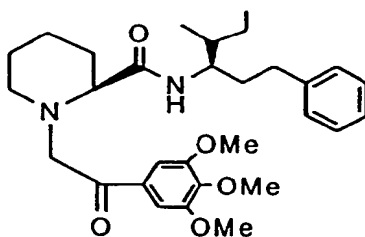
R<sub>f</sub> = 0.63 (30% EtOAc in hexane).

b) *trans* 1-Phenyl-3-(S)-amino-4-(S)-methylhexa-1-ene. A solution of *trans* 1-phenyl-3-(S)-[[[(1,1-dimethylethoxy)carbonyl]-amino]-4-(S)-methylhexa-1-ene] (8.7 g, 30.27 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (50 mL) and treated with trifluoroacetic acid (20 mL). After 20 min stirring at 22 °C, the reaction appeared complete (TLC). The reaction mixture was neutralized with excess sat. aq. NaHCO<sub>3</sub>, washed with sat. aq. NaCl (20 mL), dried (MgSO<sub>4</sub>) and evaporated to a residue. The resulting colorless oil was dissolved in Et<sub>2</sub>O (100 mL) and extracted with 1 N HCl (3 x 50 mL). The aqueous layer was neutralized with 1 N NaOH and extracted with Et<sub>2</sub>O (3 x 50 mL). The organic layer was dried (MgSO<sub>4</sub>) and concentrated in vacuo to provide 2.8 g (50%) of the title compound as a colorless oil that solidified on standing. Th <sup>1</sup>H

NMR and Mass spectrum analysis of this compound was consistent with the structure.  $R_f = 0.04$  (30% EtOAc in hexane).

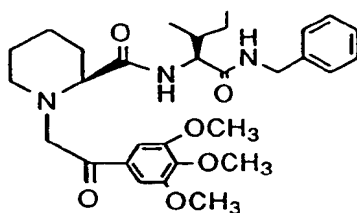
c) **L-Homoproline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] (1-(S)-[2'-(S)-methylpropyl]-3-phenylprop-2-E-enyl)-amide.** Following the procedure described in Example 111b, the coupling of N-[2-(3,4,5-trimethoxyphenyl)-2-oxoethyl]-L-homoproline hydrochloride (300 mg, 0.80 mmol) and trans 1-phenyl-3-(S)-amino-4-(S)-methylhexa-1-ene (228 mg, 1.2 mmol) provided 280 mg of the title compound as a solid. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure. LSIMS = 509 (mass calculated for  $\text{C}_{30}\text{H}_{40}\text{N}_2\text{O}_5 = 508.66$ ).

#### Example 144



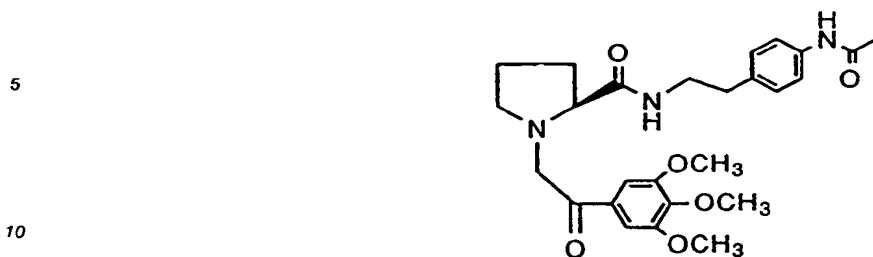
**L-Homoproline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] (1-(S)-[2'-(S)-methylpropyl]-3-phenylpropyl)-amide.** In a round bottomed flask equipped with a magnetic stirrer was added L-homoproline, 1-[2-(3,4,5-trimethoxyphenyl)-2-oxoethyl] (1-(S)-[2'-(S)-methylpropyl]-3-phenylprop-2-enyl)-amide (220 mg, 0.43 mmol), 10% palladium on carbon (22 mg), and methanol (50 mL). The flask was purged with hydrogen and the slurry stirred under an atmosphere of  $\text{H}_2$  for 2 hours. The catalyst was removed by filtration through celite and the solvent removed in vacuo to provide 220 mg of the title compound. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure. LSIMS = 511 (mass calculated for  $\text{C}_{30}\text{H}_{42}\text{N}_2\text{O}_5 = 510.68$ ).

#### Example 145



**L-Isoleucine, N-[1-(2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl)-L-Homopropyl] Benzylamide.** Following the procedure described in Example 111b, the coupling of N-[2-(3,4,5-trimethoxyphenyl)-2-oxoethyl]-L-homoproline hydrochloride (200 mg, 0.53 mmol) and L-isoleucine benzylamide (118 mg, 0.53 mmol) provided 190 mg of the title compound as a solid. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure. LSIMS = 540 (mass calculated for  $\text{C}_{30}\text{H}_{41}\text{N}_3\text{O}_6 = 539.68$ ).



**Example 146**

**L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 2-(4-(N-Acetyl)aminophenyl)ethylamide.** In an oven-dried round bottomed flask was added L-proline, 1-[2-(3,4,5-trimethoxyphenyl)-2-oxoethyl] 2-(4-aminophenyl)ethylamide (100 mg, 0.23 mmol), and tetrahydrofuran (5 mL). The solution was stirred at 22 °C, and treated with pyridine (0.037 mL, 0.45 mmol, 2.0 eq) followed by acetyl chloride (0.024 mL, 0.34 mmol, 1.5 eq). The reaction mixture was allowed to stir for one hour. The solvent was removed in vacuo and the residue partitioned between EtOAc (50 mL) and sat. NaHCO<sub>3</sub> (50 mL). The organic layer was washed with sat. aq. NaCl, dried (MgSO<sub>4</sub>) and concentrated to an oil. The oil was purified by flash chromatography to provide 70 mg of the title compound as a foam. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure.

LSIMS = 484 (mass calculated for C<sub>26</sub>H<sub>33</sub>N<sub>3</sub>O<sub>6</sub> = 483.57).

**Example 147**

**L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 2-(4-(N-Benzoyl)aminophenyl)ethylamide.** Following the procedure described in Example 146, treatment of L-proline, 1-[2-(3,4,5-trimethoxyphenyl)-2-oxoethyl] 2-(4-aminophenyl)ethylamide (100 mg, 0.23 mmol) with benzoyl chloride (0.039 mL, 0.34 mmol) provided 67 mg of the title compound as a foam. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure.

R<sub>f</sub> = 0.35 (EtOAc)

LSIMS = 546 (mass calculated for C<sub>31</sub>H<sub>35</sub>N<sub>3</sub>O<sub>6</sub> = 545.64).

**Example 148**

**L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 2-(4-(N-carboalloxy)aminophenyl)ethylamide.** Following the procedure described in example 146, treatment of L-proline, 1-[2-(3,4,5-trimethoxyphenyl)-2-oxoethyl] 2-(4-aminophenyl)ethylamide (100 mg, 0.23 mmol) with allyl chloroformate (0.036 mL, 0.34 mmol) provided 90 mg of the title compound as a foam. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure.

R<sub>f</sub> = 0.42 (EtOAc)

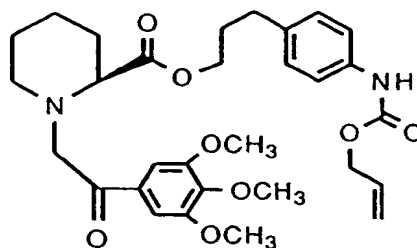
LSIMS = 526 (mass calculated for C<sub>28</sub>H<sub>35</sub>N<sub>3</sub>O<sub>7</sub> = 525.61).

**Example 149**

**L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 2-(4-(N-Carbobenzyloxy)aminophenyl)ethylamide.** Following the procedure described in Example 146, treatment of L-proline, 1-[2-(3,4,5-trimethoxyphenyl)-2-oxoethyl] 2-(4-aminophenyl)ethylamide (80 mg, 0.18 mmol) with benzyl chloroformate (0.039 mL, 0.27 mmol) provided 72 mg of the title compound as a foam. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure.

R<sub>f</sub> = 0.42 (EtOAc)

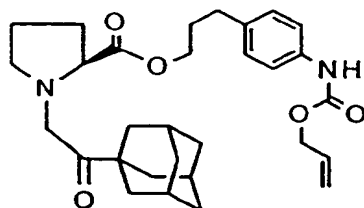
LSIMS = 577 (mass calculated for C<sub>32</sub>H<sub>37</sub>N<sub>3</sub>O<sub>7</sub> = 575.67).

**Example 150****L-Homoproline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 3-(4-(N-Carboallyloxy)aminophenyl)propyl Ester Hydrochloride.**

a) N-Carbo-*tert*-butoxy-L-homoproline 3-(4-(N-Carboallyloxy)aminophenyl)propyl Ester. To an oven-dried round bottomed flask was added N-carbotertbutoxy-L-pipecolinc acid (500 mg, 2.2 mmol), 1.0 eq benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (966 mg, 2.2 mmol), 3-(4-(N-carboallyloxy)aminophenyl)propanol (514 mg, 2.2 mmol) and anhydrous dichloromethane (20 mL). The solution was cooled to 0 °C and treated with triethylamine (0.92 mL, 6.6 mmol, 3.0 eq). After stirring for 3 hours at 0 °C, the reaction mixture was diluted with dichloromethane (100 mL), washed with 5% citric acid (100 mL), sat. NaHCO<sub>3</sub> (100 mL), sat. aq. NaCl (100 mL), and dried (MgSO<sub>4</sub>). The solution was concentrated in vacuo, and purified by flash chromatography to provide 740 mg of the title compound. This material was used directly for the next reaction.

b) L-Homoproline 3-(4-(N-Carboallyloxy)aminophenyl)propyl Ester Trifluoroacetate Salt. This material from example 150a was dissolved in ether (20 mL) and treated with trifluoroacetic acid (2 mL) and allowed to stir at 22 °C for 17 hours. The solvent was removed in vacuo and the residue triturated three times with ether and dried to provide 220 mg of L-homoproline 3-(4-(N-carboallyloxy)aminophenyl)propyl ester as the trifluoroacetic acid salt. This compound was used directly for the next reaction.

c) L-Homoproline 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 3-(4-(N-Carboallyloxy)aminophenyl)propyl Ester Hydrochloride. In an oven-dried flask, was added L-homoproline 3-(4-(N-carboallyloxy)aminophenyl)propyl ester as the trifluoroacetic acid salt (200 mg), 3,4,5-trimethoxyphenyl-2-bromoacetophenone, and THF (20 mL). The slurry was treated with triethylamine (0.3 mL 2.2 mmol, 5 eq) and heated to reflux for 4 hours. The solvent was removed in vacuo and the residue was taken up in EtOAc (100 mL) and washed with sat. NaHCO<sub>3</sub> (100 mL), sat. aq. NaCl (100 mL), dried (MgSO<sub>4</sub>) and concentrated in vacuo. The residue was purified by flash chromatography to provide the free amine (R<sub>f</sub> = 0.44: 30% EtOAc in hexane) which was treated with HCl in ether and dried in vacuo to provide 60 mg of the title compound as a solid. The 300 MHz, <sup>1</sup>H NMR analysis of this compound was consistent with the structure. LSIMS [M-HCl] = 555 (mass calculated for C<sub>30</sub>H<sub>38</sub>N<sub>2</sub>O<sub>8</sub> + HCl = 591.10).

**Example 151****L-Proline, 1-[2-Adamantan-1-yl-2-Oxoethyl] 3-(4-(N-Carboallyloxy)aminophenyl)propyl Ester Hydrochloride.**

a) N-Carbo-*tert*-butoxy-L-proline 3-(4-(N-Carboallyloxy)aminophenyl)propyl ester. Following the procedure described in Example 150a, the coupling of N-Carbo-*tert*-butoxy-L-proline (457 mg, 2.1 mmol) and

3-(4-(N-carboallyloxy)aminophenyl)propanol (500 mg, 2.1 mmol) provided 580 mg of the Boc-protected intermediate.

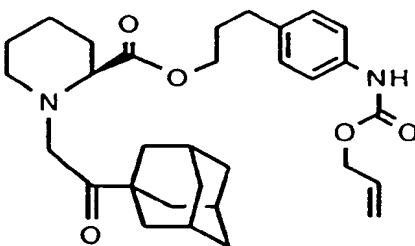
b) This intermediate from Example 151a was deprotected with 1 N HCl in ether (20 mL) and the mixture was allowed to stir for 17 hours at 22 °C. The solution was concentrated in vacuo to provide 490 mg of the corresponding hydrochloride salt.

c) L-Proline. 1-[2-Adamantan-1-yl-2-Oxoethyl] 3-(4-(N-Carboallyloxy) aminophenyl)propyl Ester Hydrochloride. A portion of the hydrochloride salt (195 mg, 0.53 mmol) was treated with 1-adamantyl  $\alpha$ -bromomethyl ketone (272 mg, 1.06 mmol), using the procedure described in Example 150c. This provided the free amine which was treated with HCl in ether and dried in vacuo to provide 90 mg of the title compound as a solid. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

Rf = 0.38 (for free base: 50% EtOAc in hexane).

LSIMS [M-HCl] = 509 (mass calculated for  $\text{C}_{30}\text{H}_{40}\text{N}_2\text{O}_5$  + HCl = 545.12).

### Example 152



**L-Homoproline, 1-[2-Adamant-1-yl-2-Oxoethyl] 3-(4-(N-Carboallyloxy)aminophenyl)propyl Ester Hydrochloride.** Following the procedure described in Example 150c, 3-(4-(N-carboallyloxy) aminophenyl)-propyl pipercolinate, trifluoroacetic acid salt (220 mg, 0.48 mmol) and 1-adamantyl  $\alpha$ -bromomethyl ketone (250 mg, 0.97 mmol) provided the amine which was treated with HCl in ether to provide 40 mg of the title compound as a powder. The 300 MHz,  $^1\text{H}$  NMR analysis of this compound was consistent with the structure.

LSIMS [M-Cl] = 523 (mass calculated for  $\text{C}_{31}\text{H}_{42}\text{N}_2\text{O}_5$  + HCl = 559.15).

The immunosuppressive properties of the present compounds were evaluated in the following assays:

#### 1) Inhibition of PPlase Activity

This assay follows in principle the procedure described in Kofron et al., 1991, Biochemistry 30:6127.

The three main reagents used are PPlase, a substrate for PPlase, and a selected inhibitor compound of the present invention. The basic principle behind this assay is the conversion of the cis isomer of the substrate to the trans form, which conversion is catalyzed by PPlase. Essentially, inhibition of this PPlase activity is measured for the selected compounds. A peptide chymotrypsin substrate containing a proline in the P2 position is only cleaved by chymotrypsin when the Phe-Pro bond is in the trans isomeric configuration. In the presence of excess chymotrypsin, all of the trans peptide isomers are cleaved within approximately five seconds, leaving only cis forms.

The cis peptide will spontaneously convert to the trans isomer at a slow rate. The cis to trans conversion is catalyzed by isomerases at a much faster rate than this spontaneous conversion. Proteins with PPlase activity are examples of such isomerases. After isomerization, the peptide is cleaved by chymotrypsin releasing p-nitroaniline which can be monitored at 390 nm. The rate of release is then calculated using a first order rate plus offset equation utilizing the ENZFITTER program (Leatherbarrow, BIOSOFT, Cambridge, United Kingdom).

**Example 153****PPlase Inhibition Assay**

In a plastic cuvette are added 950  $\mu$ l of ice cold assay buffer (25 mM HEPES, pH 7.8, 100 mM NaCl), 10  $\mu$ L of FKBP (2.5  $\mu$ M in 10 mM Tris-Cl pH 7.5, 100 mM NaCl, 1 mM dithiothreitol), 25  $\mu$ l of chymotrypsin (50 mg/ml in 1 mM HCl) and 10  $\mu$ l of the test compound at various concentrations in dimethyl sulphoxide. The reaction is initiated by addition of 5  $\mu$ l of substrate (Succinyl-Ala-Phe-Pro-Phe-para-nitroanilide, 5 mg/ml in 235 mM LiCl in trifluoroethanol).

The absorbance at 390 nm versus time is monitored for 90 sec using a Beckman DU70 spectrophotometer. The absorbance versus time data files are transferred to an IBM XT computer and the rate constants determined using the commercial Enzfitter program. For each set of data, the uncatalyzed rate of conversion is measured and the uninhibited enzymatic rate determined. The data are expressed as % Inhibition and are calculated as follows:

$$\% \text{ Inhibition} = \left[ 1 - \frac{(k_{\text{obs}} - k_{\text{uncat}})}{(k_{\text{uninh}} - k_{\text{uncat}})} \right] \times 100$$

where  $k_{\text{obs}}$  is the rate in the presence of a selected test compound,  $k_{\text{uncat}}$  is the rate in the absence of enzyme, and  $k_{\text{uninh}}$  is the rate in the presence of enzyme and absence of inhibitor. Data are plotted as percent inhibition versus concentration of inhibitor. The values of the concentration of inhibitor required for 50% inhibition of enzyme activity ( $\text{IC}_{50}$ ) were determined by nonlinear least squares regression analysis.

TABLE I

Example No.	(IC <sub>50</sub> ) $\mu$ M	Example No.	(IC <sub>50</sub> ) $\mu$ M
Example 1	2.2	Example 41	<5
Example 2	<50	Example 42	<5
Example 3	2.7	Example 43	ND
Example 4	3.4	Example 44	50
Example 5	<50	Example 45	>50
Example 6	<50	Example 46	<50
Example 7	50	Example 47	>50
Example 8	5.8	Example 48	>50
Example 9	<50	Example 49	5.1
Example 10	<50	Example 50	1.2
Example 11	<50	Example 51	0.22
Example 12	>50	Example 52	2.3
Example 13	<50	Example 53	0.71
Example 14	<50	Example 54	0.4
Example 15	<50	Example 55	<5
Example 16	<50	Example 56	1.3
Example 17	<50	Example 57	0.3
Example 18	>50	Example 58	<5
Example 19	<50	Example 59	0.1
Example 20	0.06	Example 60	0.1
Example 21	0.99	Example 61	0.71
Example 22	<50	Example 62	0.4
Example 23	12	Example 63	0.2
Example 24	<50	Example 64	0.64
Example 25	<50	Example 65	>5
Example 26	<50	Example 66	<5
Example 27	<5	Example 67	<5
Example 28	<5	Example 68	<5
Example 29	5	Example 69	<5
Example 30	<5	Example 70	>5
Example 31	>5	Example 71	<5
Example 32	2.2	Example 72	>5
Example 33	>50	Example 73	>5
Example 34	n.d.	Example 74	>5
Example 35	15	Example 75	>5
Example 36	<5	Example 76	<5
Example 37	<5	Example 77	>5
Example 38	>5	Example 78	3.2
Example 39	<5	Example 79	<5
Example 40	<5	Example 80	0.88

where ND means "not determined"

TABLE 1 (Contd.)

Example No.	(IC <sub>50</sub> ) $\mu$ M	Example No.	(IC <sub>50</sub> ) $\mu$ M
Example 81	>5	Example 117	>5
Example 82	<5	Example 118	>5
Example 83	0.12	Example 119	>5
Example 84	<5	Example 120	>5
Example 85	<5	Example 121	>5
Example 86	0.6	Example 122	>5
Example 87	1.2	Example 123	>5
Example 88	<5	Example 124	>5
Example 89	<5	Example 125	>5
Example 90	<5	Example 126	>5
Example 91	5	Example 127	>5
Example 92	>5	Example 128	>5
Example 93	<5	Example 129	>5
Example 94	5	Example 130	>5
Example 95	<5	Example 131	>5
Example 96	<5	Example 132	>5
Example 97	<5	Example 133	>5
Example 98	<5	Example 134	>5
Example 99	<5	Example 135	>5
Example 100	<5	Example 136	>5
Example 101	<5	Example 137	ND
Example 102	<5	Example 138	>5
Example 103	<5	Example 139	>5
Example 104	<5	Example 140	>5
Example 105	<5	Example 141	>5
Example 106	<5	Example 142	>5
Example 107	>5	Example 143	>5
Example 108	<5	Example 144	>5
Example 109	<5	Example 145	ND
Example 110	ND	Example 146	>5
Example 111	>5	Example 147	>5
Example 112	>5	Example 148	>5
Example 113	>5	Example 149	>5
Example 114	>5	Example 150	>5
Example 115	>5	Example 151	>5
Example 116	>5	Example 152	>5

where ND means "not determined"

Results: The results of the compound testing are presented in TABLE 1, above. As stated previously, it was not initially apparent whether or not inhibition of PPIase activity was necessary and sufficient for immunosuppression. Presently, the prevailing thought is that binding to the PPIase enzyme may be necessary but is not sufficient. Therefore, the data on PPIase inhibition may be viewed as an assay to detect whether or not a given compound is capable of interacting productively with FKBP.

## 2) Human T Lymphocyte Inhibition

Inhibition of mitogen-induced T-cell proliferation can be used to profile immunosuppressive activity of test compounds. In the description of the assay which follows, mitogen-induced T-cell proliferation was used

to test the inhibitory potencies of select compounds of the present invention.

In an assay similar to that described by Bradley in Mishell et al. (Eds.), 1980, Selected Methods in Cellular Immunology, pp 156-161, W.H. Freeman & Co., San Francisco, CA., T-cells were stimulated by incubation with phytohemagglutinin (PHA) which binds to cell surface molecules, including the T-cell receptor. This stimulation results in proliferation which can be measured by incorporation of [<sup>3</sup>H]-thymidine into cellular DNA.

The immunosuppressive properties of the compounds of the present invention can be determined by adding various concentrations of the compounds to these cultures and measuring the effect on T-cell proliferation.

#### **Example 154**

##### **Suppression of Human T-Cell Proliferation Assay**

Fresh LeukoPaks were obtained from the New York Blood Center, New York, NY. The cells, including erythrocytes and leukocytes, were diluted with Hank's Balanced Salt Solution (HBSS) (GIBCO, Grand Island, NY) and layered over Lymphoprep (Nycomed Pharma AS, Oslo, Norway) in sterile 50 ml conical centrifuge tubes. Lymphocytes were isolated at the Hank's/Nycomed interface after centrifugation at 2000 X g, 4 °C for 15 min. The lymphocytes were washed with Minimal Essential Medium (GIBCO) containing 2% fetal bovine serum (FBS) (Sigma Chemical Co., St. Louis, MO), 1% HEPES buffer (GIBCO) and 1% Penicillin-Streptomycin solution (GIBCO).

T-cells were further purified essentially by sheep erythrocyte (SRBC) rosetting as described by Morimoto et al., 1983, J. Immunol. 130:157. The isolated lymphocytes were adjusted to 2 X 10<sup>7</sup> cells/ml and 5 ml aliquots of the cell suspension were incubated for 10 minutes at room temperature with 5 ml of a 5% SRBC (Cappel, Organon Technika Corp., West Chester, PA) suspension. The cells were gently pelleted by centrifugation at 300 rpm for 10 minutes, followed by a 1 hour incubation at room temperature to allow rosette formation. The cells were gently resuspended, layered over Lymphoprep and centrifuged for 30 minutes at 500 X g. The pellet, containing rosetted T-cells and SRBC was treated with ice cold buffered ammonium chloride (GIBCO) to lyse the erythrocytes. T-cells were washed twice with HBSS.

Purified T-cells were resuspended at 2 X 10<sup>6</sup> cells /ml in complete culture medium composed of RPMI-1640 (Whittaker Bioproducts, Walkerville, MD) with 10% FBS (Sigma), 2 mM L-glutamine (GIBCO), 1% Penicillin-Streptomycin (GIBCO) and 15 mM HEPES (GIBCO). In 96-well plates (Becton Dickinson, Lincoln Park, NJ), 0.1 ml aliquots of T-cell suspension were mixed with 0.05 ml of 40 µg/ml PHA-M (Sigma). The compounds of this invention were dissolved in dimethylsulfoxide at 10 mM and various dilutions in complete medium were added in duplicate wells (0.05 ml/well). The plates were incubated at 37 °C in a humidified atmosphere of 5% carbon dioxide and 95% air for 72 hours.

Proliferation was assessed by measurement of [<sup>3</sup>H]-thymidine incorporation. During the last 6 hours of incubation, the cells were pulse labelled with 1µCi/well of [<sup>3</sup>H]-thymidine (New England Nuclear, Boston, MA). The cells were harvested onto glass fiber paper using a plate harvester and the radioactivity incorporated into cellular DNA corresponding to individual wells was measured by standard liquid scintillation counting methods. The mean counts per minute (CPM) of replicate wells was calculated and linear regression analysis of mean CPM versus compound concentration was used to determine the concentration of compound which would inhibit [<sup>3</sup>H]-thymidine incorporation of T-cells by 50% (IC<sub>50</sub>).

The results of this assay, presented in Table 2, are representative of the intrinsic immunosuppressive activity of the compounds of the present invention. Thus, concentrations less than 10 µ M of some of the preferred compounds suppress the T-cell proliferative response by 50%.

TABLE 2

Example No.	(IC <sub>50</sub> ) $\mu$ M
Example 1	ND
Example 2	ND
Example 3	<50
Example 4	50
Example 5	50
Example 6	<50
Example 7	34
Example 8	35
Example 9	>50
Example 10	>50
Example 11	17
Example 12	>50
Example 13	>50
Example 14	6
Example 15	18
Example 16	<11
Example 17	34
Example 18	42
Example 19	62
Example 20	12
Example 21	63
Example 22	58
Example 23	20
Example 24	ND
Example 25	ND
Example 26	ND
Example 27	32
Example 28	41
Example 29	42
Example 30	40
Example 31	43
Example 32	20
Example 33	>100
Example 34	ND
Example 35	6
Example 36	17
Example 37	6
Example 38	7
Example 39	16
Example 40	12

Example No.	(IC <sub>50</sub> ) $\mu$ M
Example 41	20
Example 42	13
Example 43	ND
Example 44	ND
Example 45	ND
Example 46	>50
Example 47	16
Example 48	ND
Example 49	13
Example 50	8
Example 51	8
Example 52	7
Example 53	10
Example 54	16
Example 55	17
Example 56	4
Example 57	6
Example 58	7
Example 59	8
Example 60	3
Example 61	19
Example 62	16
Example 63	8
Example 64	7
Example 65	>50
Example 66	ND
Example 67	>50
Example 68	<50
Example 69	37
Example 70	>50
Example 71	28
Example 72	100
Example 73	ND
Example 74	ND
Example 75	ND
Example 76	20
Example 77	7
Example 78	>100
Example 79	22
Example 80	22

where ND means "not determined"



TABLE 2 (Contd.)

Example No.	(IC <sub>50</sub> ) $\mu$ M	Example No.	(IC <sub>50</sub> ) $\mu$ M
Example 81	8	Example 117	10
Example 82	16	Example 118	>15
Example 83	8	Example 119	4
Example 84	6	Example 120	10
Example 85	4	Example 121	4
Example 86	6	Example 122	22
Example 87	6	Example 123	24
Example 88	8	Example 124	22
Example 89	12	Example 125	19
Example 90	7	Example 126	14
Example 91	>15	Example 127	7
Example 92	>15	Example 128	16
Example 93	>15	Example 129	18
Example 94	>15	Example 130	4
Example 95	>15	Example 131	10
Example 96	>15	Example 132	7
Example 97	>15	Example 133	12
Example 98	>15	Example 134	3
Example 99	1	Example 135	19
Example 100	>15	Example 136	4
Example 101	5	Example 137	ND
Example 102	6	Example 138	>15
Example 103	7	Example 139	8
Example 104	7	Example 140	>15
Example 105	8	Example 141	>15
Example 106	7	Example 142	>15
Example 107	7	Example 143	4
Example 108	6	Example 144	8
Example 109	>15	Example 145	>15
Example 110	ND	Example 146	13
Example 111	7	Example 147	9
Example 112	7	Example 148	6
Example 113	8	Example 149	5
Example 114	10	Example 150	10
Example 115	>15	Example 151	>15
Example 116	>15	Example 152	9

where ND means "not determined"

### 3) NF-AT Assay

Stimulation of T-cells leads to the appearance of several transcription factors, including one designated "NF-AT". These factors are involved in regulation of gene expression required for immunologic activation. Some of these transcription factors appear to have functions in a wide variety of cell types. By contrast, NF-AT is found primarily in T-cells and its role is restricted to early gene activation. In addition, NF-AT activity is inhibited by the immunosuppressant drugs, Cyclosporin A and FK506 (Schreiber and Crabtree, 1992, Immunology Today 13:136).

Inhibition of NF-AT activity is measured using FGL-5 cells. FGL-5 is a cloned line of stably transfected Jurkat T-cells that contain a construct in which three tandem copies of the NF-AT DNA binding site direct transcription of the lacZ gene, encoding  $\beta$ -galactosidase (Fiering et al., 1990, Genes & Development 4:1823). When these cells are stimulated with phorbol esters which activate protein kinase C and calcium ionophore to raise the intracellular calcium concentration, transcriptionally active NF-AT is produced. In T-cells, this normally leads to the expression of IL-2, T-cell growth factor. However, in FGL-5 cells NF-AT activation leads to the production of  $\beta$ -galactosidase which can be detected using an appropriate substrate.

FGL-5 cells were cultured with phorbol ester, calcium ionophore and the compounds of the present invention to measure inhibition of  $\beta$ -galactosidase activity, as shown below.

#### Example 155

##### NF-AT Inhibition Assay Directed $\beta$ -Galactosidase Expression

This assay was performed essentially as described (Bierer et al., 1990, Proc. Natl. Acad. Sci. 87:9231). FGL-5 cells were maintained in medium consisting of RPMI-1640 with 10% FBS, 2 mM L-glutamine, 1% Penicillin-Streptomycin and 15 mM HEPES buffer. The assays were done with exponentially growing cells whose density was not greater than 0.5 million cells/ml. The cells were resuspended to 3 million cells/ml in medium and 0.1 ml was added to wells of a 96-well plate.

The compounds of the present invention were dissolved in either ethanol or dimethylsulfoxide at 10 mM and 0.05 ml/well of various dilutions in medium were added to cells in duplicate wells. Treatment controls consisted of duplicate wells to which 0.05 ml/well of either medium, ethanol or dimethylsulfoxide was added. The ethanol and dimethyl sulfoxide were at the same concentration as was used for the compounds. Cells were incubated with compounds at room temperature for 10 - 15 minutes. Phorbol dibutyrate (Sigma) and ionomycin (Calbiochem) were dissolved at 50  $\mu$ g/ml and 2 mM, respectively and stored at -70 °C.

FGL-5 cells were stimulated by diluting these reagents with medium to 200 ng/ml and 8  $\mu$ M, respectively and adding of 0.05 ml/well. For unstimulated cell controls, 0.05 ml/well of medium was added to duplicate wells. The plates were incubated overnight (16-18 hours) at 37 °C in a humidified atmosphere of 5% CO<sub>2</sub> and air.

$\beta$ -galactosidase activity was measured as the fluorescence generated by the cleavage of 4-methyl umbelliferyl- $\beta$ -D-galactoside (Sigma) at the  $\beta$ -galactoside bond. After overnight incubation, the cells were centrifuged at 500 x g for 3 minutes in the 96-well plates and washed 3 times with PBS. The cells were then resuspended in 0.18 ml/well of reaction medium containing 100 mM sodium phosphate buffer, pH 7.0, 10 mM potassium chloride, 1 mM magnesium sulfate, 0.1% Triton X-100 (Pierce, Rockford, IL), and 0.5 mM 4-methylumbelliferyl- $\beta$ -D- galactoside.

The fluorescence at 460 nm using 355 nm excitation was measured at intervals over 1-2 hours (during which fluorescence increased linearly with time) with a LS50 Luminescence Spectrometer (Perkin Elmer).

The percent inhibition by each concentration of the compounds was calculated as:

$$\% \text{ Inhibition} = \frac{1 - (\text{fluorescence with compound} - \text{unstimulated control})}{(\text{fluorescence with solvent alone} - \text{unstimulated control})} \times 100$$

The values of the concentration of compounds required for 50% inhibition (IC<sub>50</sub>) were determined by linear regression analysis of the percent inhibition at various compound concentrations.

The results of this assay presented in TABLE 3 are representative of the intrinsic immunosuppressive activity of the compounds of the present invention. Compounds that inhibited NF-AT directed  $\beta$ -galactosidase expression by stimulated FGL-5 cells with IC<sub>50</sub> of 10  $\mu$ M or less also inhibited mitogen induced T-cell proliferation.

TABLE 3

Example No.	(IC <sub>50</sub> ) $\mu$ M
Example 1	ND
Example 2	ND
Example 3	ND
Example 4	ND
Example 5	ND
Example 6	ND
Example 7	ND
Example 8	ND
Example 9	ND
Example 10	ND
Example 11	ND
Example 12	ND
Example 13	ND
Example 14	ND
Example 15	ND
Example 16	ND
Example 17	ND
Example 18	ND
Example 19	ND
Example 20	ND
Example 21	ND
Example 22	ND
Example 23	ND
Example 24	ND
Example 25	ND
Example 26	ND
Example 27	ND
Example 28	ND
Example 29	ND
Example 30	ND
Example 31	ND
Example 32	13
Example 33	>100
Example 34	ND
Example 35	41
Example 36	19
Example 37	13
Example 38	22
Example 39	14
Example 40	24

Example No.	(IC <sub>50</sub> ) $\mu$ M
Example 41	>33
Example 42	15
Example 43	ND
Example 44	ND
Example 45	ND
Example 46	ND
Example 47	ND
Example 48	ND
Example 49	44
Example 50	73
Example 51	>100
Example 52	20
Example 53	>100
Example 54	18
Example 55	>100
Example 56	13
Example 57	20
Example 58	16
Example 59	17
Example 60	57
Example 61	>100
Example 62	60
Example 63	16
Example 64	16
Example 65	ND
Example 66	ND
Example 67	ND
Example 68	ND
Example 69	ND
Example 70	ND
Example 71	ND
Example 72	ND
Example 73	ND
Example 74	ND
Example 75	ND
Example 76	ND
Example 77	12
Example 78	>100
Example 79	13
Example 80	13

where ND means "not determined"

TABLE 3 (Contd.)

Example No.	(IC <sub>50</sub> ) $\mu$ M	Example No.	(IC <sub>50</sub> ) $\mu$ M
Example 81	6	Example 117	10
Example 82	13	Example 118	>15
Example 83	5	Example 119	4
Example 84	>100	Example 120	>15
Example 85	26	Example 121	>15
Example 86	6	Example 122	>33
Example 87	6	Example 123	>33
Example 88	8	Example 124	>33
Example 89	12	Example 125	>33
Example 90	7	Example 126	>33
Example 91	>15	Example 127	>33
Example 92	>15	Example 128	>33
Example 93	>15	Example 129	>33
Example 94	>15	Example 130	>33
Example 95	>15	Example 131	>33
Example 96	>15	Example 132	>33
Example 97	>15	Example 133	>33
Example 98	>15	Example 134	>33
Example 99	1	Example 135	>33
Example 100	>15	Example 136	>33
Example 101	5	Example 137	ND
Example 102	6	Example 138	>15
Example 103	7	Example 139	>15
Example 104	7	Example 140	>15
Example 105	8	Example 141	>15
Example 106	7	Example 142	>15
Example 107	7	Example 143	>15
Example 108	6	Example 144	>15
Example 109	>15	Example 145	>15
Example 110	ND	Example 146	>15
Example 111	7	Example 147	>15
Example 112	7	Example 148	>15
Example 113	8	Example 149	>15
Example 114	10	Example 150	3
Example 115	>15	Example 151	>15
Example 116	>15	Example 152	>15

where ND means "not determined"

#### 4) Graft versus Host Assay

Inhibition of the graft versus host response (hereinafter "GVHR") by the compounds of the present invention is another means to demonstrate their immunosuppressive activity. Transfer of parental strain T-cells (the graft) into F1 hybrid animals (the host) different with respect to gene products of the major histocompatibility complex (MHC) causes a GVHR. This reaction results from recognition of host allogeneic MHC gene products by specific clones of graft T-cells.

When given systemically in sufficient numbers, the graft T-cells cause a progressive, generally fatal, wasting syndrome. A local, nonfatal GVHR, marked by enlargement of the draining popliteal lymph nodes, ensues when graft T-cells are administered via the footpad as described by Ford et al., 1970, Transplantation 10:258. The GVHR is regarded as a correlate of allograft rejections where specific T-cells of either host or allograft origin are activated after recognition of allogeneic MHC gene products, leading to an immune inflammatory response which ultimately results in the destruction (rejection) of the allograft.

### Example 156

#### 10 Mouse Lymph Node Assay for Modulation of Graft versus Host Response

Single cell suspensions in phosphate buffered saline (PBS) were prepared from the spleens of BDF1 and C57Bl/6 mice (Jackson Labs, Bar Harbor, ME). The cells were pelleted by centrifugation at 500 X g for 5 minutes and the pellet resuspended in 0.9 ml distilled water to lyse erythrocytes. After 5 seconds, 0.1 ml 10X concentrated PBS was added, resulting in an isotonic solution. The cells were washed with PBS and resuspended at  $2 \times 10^8$  cells/ml.  $1 \times 10^7$  cells in 0.05 ml PBS were injected subcutaneously into the hind footpads (BDF1 cells in one footpad, C57Bl/6 cells in the other). The test compounds were dissolved in ethanol, mixed with olive oil (1:7, ethanol:olive oil). Some mice received intraperitoneal injections (0.2 ml/injection) of either ethanol:olive oil alone (vehicle control group) or compound at 100 mg/kg per day, beginning on the same day as the spleen cell injections.

After 7 days, the draining popliteal lymph nodes from the hind limbs were dissected out and weighed. The magnitude of the GVHR was expressed as the ratio of the mean weight of lymph nodes from the limb injected with semi-allogeneic C57Bl/6 cells divided by the mean weight of lymph nodes from the limb injected with syngeneic BDF1 cells.

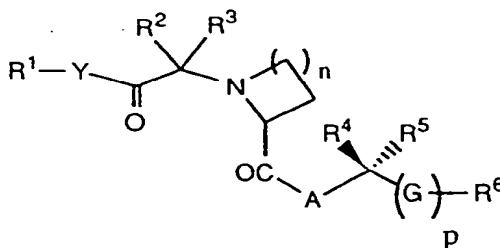
The results presented in TABLE 4 show that a representative compound of this invention which is a potent inhibitor of both FKBP activity and mitogen-induced T-cell proliferation also inhibited the localized GVHR. Thus, for the untreated or vehicle control groups, the mean lymph node weights from BDF1-sensitized limbs were 2.2 - 3.1 times that of C57Bl/6-sensitized limbs. By contrast, in mice treated with the test compound, virtually no GVHR was detected (ratio=1.2). For comparison, in this example a group of mice was treated with 100 mg/kg/day of cyclosporin A (Sandoz Ltd., Basel, Switzerland). Cyclosporin A also inhibited the GVHR (ratio=1.6).

TABLE 4

Treatment	Lymph Node Weight Ratio
None	3.1
Vehicle alone	2.2
Compound of Example 16	1.2
Cyclosporin A	1.6

### Claims

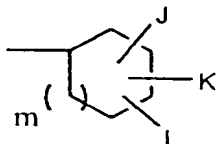
1. A compound of the following structure



wherein

R<sup>1</sup> is

- a) hydrogen,
- b) linear or branched alkyl (C1-C8) which may be substituted independently or simultaneously up to two times by
  - i) hydroxy,
  - ii) phenyl which may be substituted by straight or branched alkyl (C1-C8), or straight or branched alkoxy (C1-C6),
  - iii) cycloalkyl (C3-C10) which may be substituted by straight or branched alkyl (C1-C8), or straight or branched alkoxy (C1-C6),
  - iv) bicycloalkyl (C6-C12) which may be substituted by straight or branched alkyl (C1-C10), or straight or branched alkoxy (C1-C6),
  - v) tricycloalkyl (C7-C14) which may be substituted by straight or branched alkyl (C1-C8), or straight or branched alkoxy (C1-C6),
  - vi) tetracycloalkyl (C10-C14), which may be substituted by straight or branched alkyl (C1-C8), or straight or branched alkoxy (C1-C6), or
  - vii) morpholinyl,
- c) alkene (C3-C10), diene (C4-C10), or triene (C8-C18), which may be substituted independently or simultaneously up to three times by
  - i) phenyl,
  - ii) straight or branched alkyl (C1-C6), or
  - iii) straight or branched alkoxy (C1-C6),
- d) cycloalkyl (C5-C10), or the cycloalkyl fragment

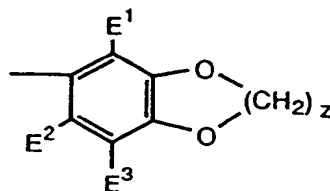


where

m is an integer of 0, 1, or 2,

J, K, and L are independently or simultaneously

- i) hydrogen,
- ii) straight or branched alkyl (C1-C5), which may be substituted by phenyl, or straight or branched alkoxy (C1-C6),
- iii) straight or branched alkoxy (C1-C5),
- iii) phenyl, or
- iv) phenyl substituted by straight or branched alkyl (C1-C6), or chlorine, or straight or branched alkoxy (C1-C6),
- e) bicycloalkyl (C7-10), tricycloalkyl (C7-14), tetracycloalkyl (C10-C16), or pentacycloalkyl (C11-C20), which may be independently or simultaneously substituted up to 3 times with straight or branched alkyl (C1-C6), or straight or branched alkoxy (C1-C6), or phenyl,
- f) the aryl derivatives tetrahydronaphthyl, benzothienyl, benzofuryl, benzopyranyl, furyl, pyridyl, pyranyl, 1,3-oxazolyl, or naphthyl, said aryl derivatives may be independently or simultaneously substituted up to two times by
  - i) straight or branched alkyl (C1-C6),
  - ii) straight or branched alkoxy (C1-C6),
  - iii) halogen, where halogen is fluoro, chloro, bromo, or iodo,
- g) the piperonyl fragment

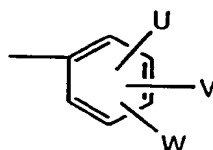


where

z is an integer of 1, or 2,

and E¹, E², and E³ can independently or simultaneously be hydrogen, straight or branched alkyl (C1-C6), straight or branched alkoxy (C1-C6), or chlorine, or

h) the aryl derivative



where

U, V, and W can be independently or simultaneously

i) hydrogen,

ii) straight or branched alkyl (C1-C6), straight or branched alkoxy (C1-C6), phenyl, or phenoxy, these groups may be substituted by phenyl, straight or branched alkoxy (C1-C6), or phenoxy,

iii) hydroxy,

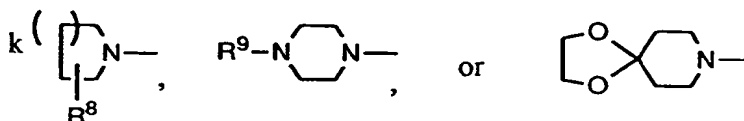
iv) halogen,

v) nitro, or

vi) benzoyl;

Y is a covalent bond, oxygen, NR⁷, where R⁷ is hydrogen, in addition,

R¹—Y— may also be



where

k is an integer of 1 or 2

R⁸ is

a) hydrogen,

b) carboalkoxy with a straight or branched alkoxy (C1-C6),

c) straight or branched alkyl (C1-C6) which may be substituted by phenyl, or straight or branched alkoxy (C1-C6),

d) phenyl, or phenyl substituted by halogen,

R⁹ is phenyl which may be substituted by straight or branched alkyl (C1-C6);

R² and R³ are defined as follows: one of R² and R³ are hydrogen, and the other is hydrogen or straight or branched alkyl (C1-C6);

n is an integer of 2 or 3;

A is oxygen or NR¹⁰, where R¹⁰ is hydrogen or straight or branched alkyl (C1-C6);

R⁴ and R⁵ may independently or simultaneously be

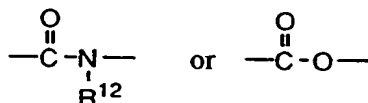
a) hydrogen,

b) straight or branched alkyl (C1-C8) which may be substituted by

i) phenyl, or phenyl substituted by hydroxy or alkoxy (C1-C2),

ii) cycloalkyl (C5-C6),

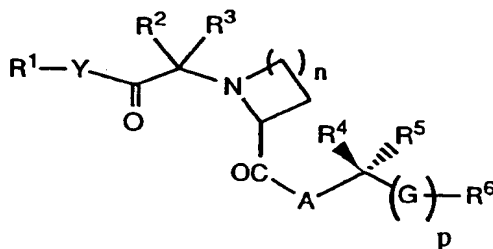
- iii) alkylthio (C1-C6),  
 iv) carboxamido,  
 v) straight or branched alkoxy (C1-C6) which may be substituted by phenyl,  
 c) phenyl, or  
 d) cycloalkyl (C3-C7), which may be substituted by straight or branched alkyl (C1-C6),  
 in addition, R<sup>4</sup> and R<sup>5</sup>, taken together can be  
 -(CH<sub>2</sub>)<sub>r</sub>--  
 where  
 r is an integer of 4 or 5;  
 G is one of the following fragments  
 -HC=CH-, -CH<sub>2</sub>-CH<sub>2</sub>-, or -CH<sub>2</sub>-  
 or one of the following fragments



where R<sup>12</sup> is hydrogen or methyl, such that the carbonyl group is attached to the carbon bearing R<sup>4</sup> and R<sup>5</sup> and that NR<sup>12</sup> and oxygen are connected to R<sub>6</sub>, respectively;  
 p is an integer of 0 or 1;  
 R<sup>6</sup> is

- a) hydrogen,  
 b) straight or branched alkyl (C1-C6) which may be substituted by  
 i) phenyl,  
 ii) phenyl substituted with straight or branched alkyl (C1-C6), straight or branched alkoxy (C1-C6),  
 or  
 iii) pyridyl, or  
 c) phenyl, naphthyl, furyl, thiofuryl, cycloalkyl (C5-C8), bicycloalkyl (C6-C10), tricycloalkyl (C7-C12),  
 tetracycloalkyl (C10-C16), pentacycloalkyl (C11-C20) or benzoyl, such groups may be substituted by  
 i) an amine,  
 ii) amino substituted by a straight or branched alkoxy carbonyl (C1-C6) that may be substituted by  
 phenyl or an alkene (C2-C6),  
 iii) amino substituted by alkanoyl (C1-C6), or benzoyl,  
 iv) sulfonamide (-SO<sub>2</sub>NH<sub>2</sub>), or  
 v) hydroxy, or a straight or branched alkoxy (C1-C6), that may be substituted by phenyl;  
 and said composition's pharmaceutically acceptable salts.

## 2. A compound of the following structure



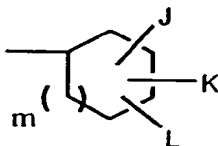
wherein

R<sup>1</sup> is

- a) hydrogen,  
 b) linear or branched alkyl (C1-C6) which may be substituted



- i) once by hydroxy,  
 ii) once by phenyl which may be substituted by straight or branched alkyl (C1-C4), or straight or branched alkoxy (C1-C6),  
 iii) once by cycloalkyl (C3-C8) which may be substituted by straight or branched alkyl (C1-C6), or straight or branched alkoxy (C1-C4),  
 iv) once by bicycloalkyl (C6-C10) which may be substituted by straight or branched alkyl (C1-C8), or straight or branched alkoxy (C1-C4),  
 v) once by tricycloalkyl (C7-C12) which may be substituted by straight or branched alkyl (C1-C6), or straight or branched alkoxy (C1-C4),  
 vi) once by tetracycloalkyl (C10-C12), which may be substituted by straight or branched alkyl (C1-C8), or straight or branched alkoxy (C1-C4),  
 vii) up to two times by phenyl and cycloalkyl (C5-C7), or  
 viii) up to two times by phenyl and morpholinyl,  
 c) alkene (C3-C8), which may be substituted by phenyl, straight or branched alkyl (C1-C4), or straight or branched alkoxy (C1-C4),  
 d) diene (C4-C7) substituted by straight or branched alkyl (C1-C6), or straight or branched alkoxy (C1-C4),  
 e) triene (C10-C16) substituted up to three times by straight or branched alkyl (C1-C6), or straight or branched alkoxy (C1-C4), or  
 f) cycloalkyl (C5-C10), or the cycloalkyl fragment

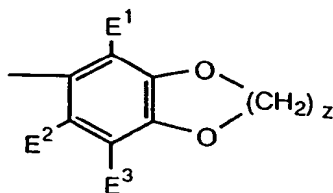


where

m is an integer of 0, 1, or 2,

J, K, and L are independently or simultaneously

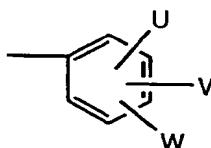
- i) hydrogen,  
 ii) straight or branched alkyl (C1-C5), which may be substituted by phenyl, or straight or branched alkoxy (C1-C4),  
 iii) phenyl, or  
 iv) phenyl substituted by straight or branched alkyl (C1-C4), or chlorine, or straight or branched alkoxy (C1-C4),  
 g) bicycloalkyl (C7-10) which may be substituted up to 3 times with straight or branched alkyl (C1-C6), or straight or branched alkoxy (C1-C4),  
 h) tricycloalkyl (C7-14) which may be substituted up to 3 times with straight or branched alkyl (C1-C6), or straight or branched alkoxy (C1-C4),  
 i) tetracycloalkyl (C10-C15) which may be substituted up to 3 times by straight or branched alkyl (C1-C6), or straight or branched alkoxy (C1-C4),  
 j) naphthyl derivatives, or the heteroaryl derivatives benzothienyl, benzofuryl, benzopyranyl, furyl, pyridyl, pyranal, or 1,3-oxazolyl, said derivatives may be substituted up to two times by  
 i) straight or branched alkyl (C1-C6),  
 ii) halogen,  
 iii) or both,  
 k) 1,2,3,4-tetrahydronaphthyl,  
 l) the piperonyl fragment



where

z is an integer of 1, or 2,

and E¹, E², and E³ can be independently or simultaneously hydrogen, straight or branched alkyl (C1-C4), straight or branched alkoxy (C1-C4), or chlorine, m) the aryl derivative



where

U, V, and W can be independently or simultaneously

i) hydrogen,

ii) straight or branched alkyl (C1-C4), which may be substituted by phenyl,

iii) straight or branched alkoxy (C1-C6) which may be substituted by phenyl, straight or branched alkoxy (C1-C4), or phenoxy,

iv) hydroxy,

v) phenyl,

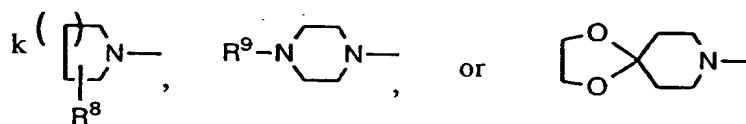
vi) halogen,

vii) nitro,

viii) benzoyl, or

ix) phenoxy;

Y is a covalent bond, oxygen, NR⁷, where R⁷ is hydrogen, in addition, R¹—Y— may also be



where

k is an integer of 1, or 2,

R⁸ is

a) hydrogen,

b) carboalkoxy with a straight or branched alkoxy (C1-C4),

c) straight or branched alkyl (C1-C4) which may be substituted by phenyl, or straight or branched alkoxy (C1-C4), or

d) phenyl, or phenyl substituted by halogen,

R⁹ is phenyl which may be substituted by alkyl (C1-C4);

R² and R³ are defined as follows: one of R² and R³ are hydrogen, and the other is hydrogen or straight or branched alkyl (C1-C6);

n is an integer of 2 or 3;

A is oxygen or NR¹⁰, where R¹⁰ is hydrogen or straight or branched alkyl (C1-C4);

R<sup>4</sup> is

- a) hydrogen,
- b) straight or branched alkyl (C1-C6) which may be substituted by
  - i) phenyl, or phenyl substituted by hydroxy or methoxy,
  - ii) cycloalkyl (C5-C6),
  - iii) alkylthio (C1-C6),
  - iv) carboxamido, or
  - v) straight or branched alkoxy (C1-C6) which may be substituted by phenyl,
- c) phenyl, or

- d) cycloalkyl (C3-C7), which may be substituted by straight or branched alkyl (C1-C6);

R<sup>5</sup> is hydrogen or straight or branched alkyl (C1-C4), and R<sup>4</sup> and R<sup>5</sup>, taken together can be

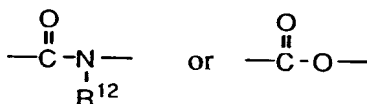
-(CH<sub>2</sub>)<sub>r</sub>-

where r is an integer of 4 or 5;

G is one of the following fragments

-HC=CH-, -CH<sub>2</sub>-CH<sub>2</sub>-, or -CH<sub>2</sub>-

or one of the following fragments



where the carbonyl group is attached to the carbon bearing R<sup>4</sup> and R<sup>5</sup> and NR<sup>12</sup> and oxygen are connected to R<sup>6</sup>, respectively,

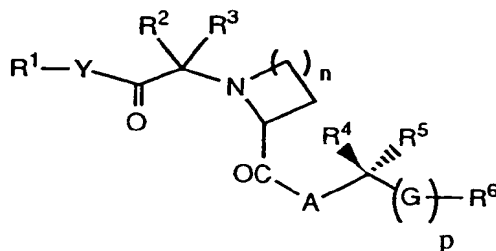
R<sup>12</sup> is hydrogen or methyl;

p is an integer of 0 or 1;

R<sup>6</sup> is

- a) hydrogen,
  - b) straight or branched alkyl (C1-C4) which may be substituted by
    - i) phenyl,
    - ii) phenyl substituted with straight or branched alkyl (C1-C4), straight or branched alkoxy (C1-C4), or
    - iii) 2- or 4-pyridyl,
  - c) phenyl or naphthyl, which may be substituted by
    - i) amine,
    - ii) amino substituted by a straight or branched alkoxycarbonyl (C1-C6) that may be substituted by phenyl or an alkene (C2-C6),
    - iii) amino substituted by alkanoyl (C1-C6), or benzoyl,
    - iv) sulfonamide (-SO<sub>2</sub>NH<sub>2</sub>), or
    - v) straight or branched alkoxy (C1-C6), that may be substituted by phenyl,
  - d) benzoyl,
  - e) furyl, or thiofuryl, or
  - f) cycloalkyl (C5-C8), bicycloalkyl (C6-C10), tricycloalkyl (C7-C12), or tetracycloalkyl (C10-C14);
- and said composition's pharmaceutically acceptable salts.

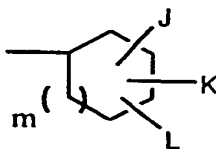
### 3. A compound of the following structure



wherein

R<sup>1</sup> is

- a) hydrogen,
- b) linear or branched alkyl (C1-C6) which may be substituted by
  - i) hydroxy,
  - ii) phenyl, or phenyl substituted by straight or branched alkyl (C1-C4),
  - iii) cycloalkyl (C3-C8) which may be substituted by straight or branched alkyl (C1-C4),
  - iv) bicycloalkyl (C6-C9) which may be substituted by straight or branched alkyl (C1-C6),
  - v) tricycloalkyl (C7-C12) which may be substituted by straight or branched alkyl (C1-C4),
  - vi) tetracycloalkyl (C10-C12), which may be substituted by straight or branched alkyl (C1-C6),
  - vii) both phenyl and cycloalkyl (C5-C6), or
  - viii) both phenyl and morpholiny,
- c) alkene (C3-C6), which may be substituted by phenyl,
- d) diene (C5-C6) substituted by straight or branched alkyl (C1-C4),
- e) triene (C13-C16) substituted up to three times by straight or branched alkyl (C1-C4),
- f) cycloalkyl (C5-C6), or the cycloalkyl fragment

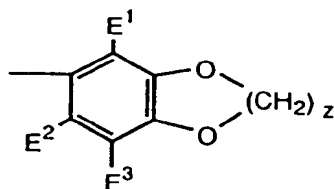


where

m is an integer of 0, 1, or 2,

J, K, and L are independently or simultaneously

- i) hydrogen,
- ii) straight or branched alkyl (C1-C5),
- iii) phenyl, or
- iv) phenyl substituted by straight or branched alkyl (C1-C4), or chlorine, or straight or branched alkoxy (C1-C4),
- g) bicycloalkyl (C7-8) which may be substituted up to 3 times with straight or branched alkyl (C1-C4),
- h) tricycloalkyl (C7-12) which may be substituted up to 2 times with straight or branched alkyl (C1-C6),
- i) tetracycloalkyl (C10-C12) which may be substituted up to 3 times by straight or branched alkyl (C1-C4),
- j) 2-benzothieryl substituted independently or simultaneously at least twice by either
  - i) straight or branched alkyl (C1-C3),
  - ii) chlorine,
  - iii) or both,
- k) 2-furyl,
- l) 2-pyridyl,
- m) 2-naphthyl,
- n) 1,2,3,4-tetrahydronaphthyl,
- o) 2-benzopyranyl,
- p) 2-benzofuryl,
- q) the piperonyl fragment

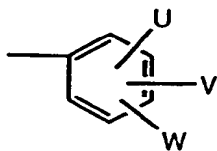


where

z is an integer of 1, or 2,

and E<sup>1</sup>, E<sup>2</sup>, and E<sup>3</sup> are hydrogen, or

r) the aryl derivative



where

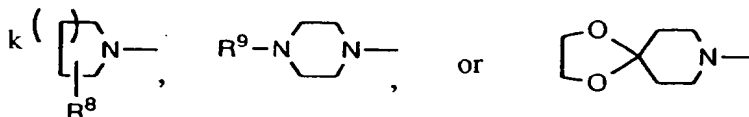
U, V, and W can be independently or simultaneously

- i) hydrogen,
- i) straight or branched alkyl (C1-C4),
- ii) straight or branched alkoxy (C1-C4),
- iii) alkoxy (C2) substituted by alkoxy (C2), or phenoxy,
- iv) hydroxy,
- v) phenyl,
- vi) fluorine,
- vii) chlorine,
- viii) bromine,
- ix) nitro,
- x) benzyloxy,
- xi) benzoyl,
- xii) phenoxy;

Y is a covalent bond, oxygen, NR<sup>7</sup>, where R<sup>7</sup> is hydrogen;

in addition,

R<sup>1</sup>-Y- may also be



where

k is an integer of 1, or 2,

R<sup>8</sup> is

- a) hydrogen,
- b) carboalkoxy with alkoxy (C1-C2),
- c) straight or branched alkyl (C1-C4) which may be substituted by phenyl,
- d) phenyl,

R<sup>9</sup> is phenyl;

R<sup>2</sup> and R<sup>3</sup> are defined as follows: one of R<sup>2</sup> and R<sup>3</sup> is hydrogen, and the other is hydrogen or straight or branched alkyl (C1-C4);

n is an integer of 2 or 3;

A is oxygen or NR<sup>10</sup>, where R<sup>10</sup> is hydrogen or methyl;

R<sup>4</sup> is

- a) hydrogen,
- b) straight or branched alkyl (C1-C4) which may be substituted by
  - i) phenyl,
  - ii) cycloalkyl (C5-C6),
  - iii) alkylthio (C1-C4),
  - iv) carboxamido, or
  - v) benzyloxy, or

c) phenyl;

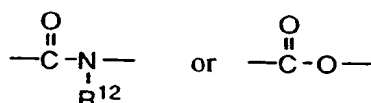
R<sup>5</sup> is hydrogen or straight or branched alkyl (C1-C4), and R<sup>4</sup> and R<sup>5</sup>, taken together can be  
-(CH<sub>2</sub>)<sub>r</sub>-

where r is integer 5;

G is one of the following fragments

-HC=CH-, -CH<sub>2</sub>•CH<sub>2</sub>-, or -CH<sub>2</sub>-

or one of the following fragments



where the carbonyl group is attached to the carbon bearing R<sup>4</sup> and R<sup>5</sup> and NR<sup>12</sup> and oxygen are  
connected to R<sup>6</sup>, respectively,

R<sup>12</sup> is hydrogen or methyl;

p is an integer of 0 or 1;

R<sup>6</sup> is

a) hydrogen,

b) straight or branched alkyl (C1-C4) which may be substituted by

i) phenyl,

ii) phenyl substituted with alkoxy (C1-C2),

iii) 2- or 4-pyridyl,

c) phenyl which may be substituted by

i) amino,

ii) amino substituted by allyloxycarbonyl,

iii) amino substituted by acetyl,

iv) amino substituted by benzoyl,

v) amino substituted by benzyloxycarbonyl,

iii) sulfonamide (-SO<sub>2</sub>NH<sub>2</sub>), or

iv) straight or branched alkoxy (C1-C4),

d) benzoyl,

e) furyl,

f) naphthyl,

g) cycloalkyl (C5-C8), or

h) tetracycloalkyl (C10-C12);

and said composition's pharmaceutically acceptable salts.

4. A compound according to Claim 1 for the treatment of inflammations.

5. The compound of claim 3 comprising a composition represented by a structure selected from the  
group consisting of:

L-Isoleucine, N-[1-(2-Benzyloxy-2-Oxoethyl)-L-Prolyl] Benzylamide;

L-Isoleucine, N-[1-(2-Methoxy-2-Oxoethyl)-L-Prolyl] Benzylamide;

L-Isoleucine, N-[1-(2-Phenyl-2-Oxoethyl)-L-Prolyl] Benzylamide;

L-Isoleucine, N-[1-(2-Naphth-2-yl-2-Oxoethyl)-L-Prolyl] Benzylamide;

L-Isoleucine, N-[1-(2-(Biphenyl-4-yl)-2-Oxoethyl)-L-Prolyl] Benzylamide;

L-Isoleucine, N-[1-(2-(2-Methoxyphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide;

L-Isoleucine, N-[1-(2-(5-Chloro-3-Methyl-benzo[B]thiophene-2-yl)-2-Oxoethyl)-L-Prolyl] Benzylamide;

L-Isoleucine, N-[1-(2-(trans,trans-Hexa-2,4-dienyl-1-oxy)-2-Oxoethyl)-L-Prolyl] Benzylamide;

L-Isoleucine, N-[1-(2-(4-Chlorophenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide;

L-Isoleucine, N-[1-(2-(4-Methylphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide;

L-Isoleucine, N-[1-(2-(4-Methoxyphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide;

L-Isoleucine, N-Methyl-N-[1-(2-Phenyl-2-Oxoethyl)-L-Prolyl] Benzylamide;

L-Isoleucine, N-[1-(2-Phenyl-2-Oxoethyl)-L-Homoproline] Benzylamide;

L-Phenylglycine, N-[1-(2-Phenyl-2-Oxoethyl)-L-Proline] Benzylamide;

L-Isoleucine, N-[1-(1-Methyl-2-Phenyl-2-Oxoethyl)-L-Prolyl] Benzylamide;

L-Isoleucine, N-[1-(2-(3-Methoxyphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide;

- L-Isoleucine, N-[1-(2-(3,4-Dihydroxyphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-Methyl-N-[1-(2-Benzoyloxy-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(Carbobenzoyloxymethylene)-L-Homoproline] Benzylamide;  
 L-Isoleucine, N-[1-(2-Adamantan-1-yl-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 5 L-Isoleucine, N-[1-(Carbo-tert-Butoxymethylene)-L-Proline] Benzylamide;  
 L-Isoleucine, N-[1-(2-tert-Butyl-2-Oxoethyl)-L-Proline] Benzylamide;  
 L-Isoleucine, N-[1-(2-(2,5-Dimethoxyphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-(2,4-Dimethoxyphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-(2-Nitrophenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 10 L-Isoleucine, N-[1-(2-(4-Nitrophenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-(3-Benzoyloxyphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-(2,4-Dimethylphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-(4-Fluorophenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-(4-Bromophenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 15 L-Isoleucine, N-[1-(2,4-Dichlorophenylcarbamoylmethyl)-L-Proline] Benzylamide;  
 L-Isoleucine, N-[1-(2-Adamantan-1-yl-2-Oxoethyl)-L-Homoproline] Benzylamide;  
 L-Isoleucine, N-[1-(2-Furan-2-yl-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-Pyrid-2-yl-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(Adamant-1-ylcarbamoylmethyl)-L-Prolyl] Benzylamide;  
 20 L-Isoleucine, N-[1-(2-(cis-Octahydro-pentalen-1-yl)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-(2,6,6-Trimethyl-Bicyclo[3.1.1]hept-3-yl)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-(4-Pentylcyclohexyl)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-(1,2,3,4-tetrahydro-Napththalen-2-yl)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-(1-Methyl-Cyclohexyl)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 25 L-Isoleucine, N-[1-(2-Oxo-2-Tricyclo[3.3.1.0<sup>3,7</sup>]Non-3-yl-Ethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-Oxo-3-(3-Methyl-Adamantan-1-yl)-Propyl)-L-Prolyl] Benzylamide;  
 L-Proline, 1-(2-Adamantan-1-yl-2-Oxoethyl) Benzyl Ester;  
 L-Isoleucine, N-[1-(2-(Biphenyl-4-yl)-2-Oxoethyl)-L-Prolyl] 1,2,3,4-Tetrahydroisoquinolinamide;  
 L-Isoleucine, N-[1-(2-(Biphenyl-4-yl)-2-Oxoethyl)-L-Prolyl] Benzyl Ester;  
 30 L-Isoleucine, N-[1-(2-(Biphenyl-4-yl)-2-Oxoethyl)-L-Prolyl] tert-Butylamide;  
 L-Phenylalanine, N-[1-(2-(Biphenyl-4-yl)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Methionine, N-[1-(2-(Biphenyl-4-yl)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 Glycine, N-[1-(2-Adamantan-1-yl-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Valine, N-[1-(2-Adamantan-1-yl-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 35 L-Leucine, N-[1-(2-Adamantan-1-yl-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Phenylalanine, N-[1-(2-Adamantan-1-yl-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Norvaline, N-[1-(2-Adamantan-1-yl-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Norleucine, N-[1-(2-Adamantan-1-yl-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Asparagine, N-[1-(2-Adamantan-1-yl-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 40 L-Serine-(O-Benzyl Ether), N-[1-(2-Adamantan-1-yl-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L- $\beta$ -Phenylalanine, N-[1-(2-Adamantan-1-yl-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Cyclohexylalanine, N-[1-(2-Adamantan-1-yl-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-Adamantan-1-yl-2-Oxoethyl)-L-Prolyl] alpha-(S)-methylbenzylamide;  
 L-Isoleucine, N-[1-(2-Adamantan-1-yl-2-Oxoethyl)-L-Prolyl] alpha-(R)-methylbenzylamide;  
 45 L-Isoleucine, N-[1-(2-Adamantan-1-yl-2-Oxoethyl)-L-Prolyl] Pyridin-4-ylmethylamide;  
 L-Isoleucine, N-[1-(2-Adamantan-1-yl-2-Oxoethyl)-L-Prolyl] Pyridin-2-ylmethylamide;  
 L-Isoleucine, N-[1-(2-Adamantan-1-yl-2-Oxoethyl)-L-Prolyl] 4-methoxybenzylamide;  
 L-Isoleucine, N-[1-(2-Adamantan-1-yl-2-Oxoethyl)-L-Prolyl] 2-methoxybenzylamide;  
 L-Isoleucine, N-[1-(Carboxymethyl)-L-Prolyl] Benzylamide;  
 50 L-Isoleucine, N-[1-(2-[N-(Piperidine-3-Carboxylic Acid Ethyl Ester)]-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-(1,4-Dioxo-8-aza-spiro[4.5]dec-8-yl)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-(N-(4-Benzylpiperidyl))-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-(2-Methylpiperidine)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-(2-Hydroxyethylamine)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 55 L-Isoleucine, N-[1-(2-(4-Phenylpiperazine)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-(1-Pyrrolidine)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-(N-Cyclopentylamino)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-(N-(Phenylmethylamino))-2-Oxoethyl)-L-Prolyl] Benzylamide;

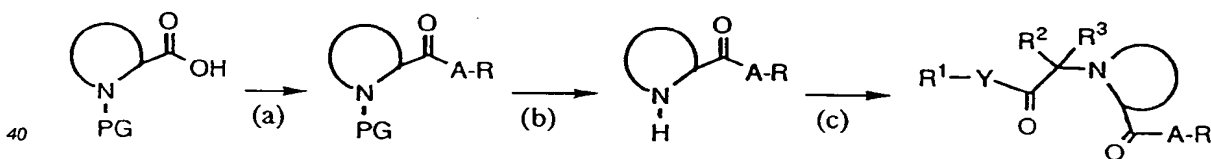
- L-Isoleucine, N-[1-(2-(N-(Cyclohexylmethylamino))-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-(4-Phenylpiperidyl)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-[1-(3,7,11-Trimethyldodeca-2,6,10-trien-1-ol)]-2-Oxoethyl)-L-Proline] Benzylamide;  
 L-Isoleucine, N-[1-(2-(3-Phenyl-2-Propen-1-Oxy)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 5 L-Isoleucine, N-[1-(2-(3-Phenyl-3-Methyl-2-Propen-1-Oxy)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-(1-Phenylpropoxy)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-(1-Phenyl-1-Cyclohexylmethoxy)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-(1-Phenyl-2-(4-Morpholino)Ethoxy)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-(2-Oxy-2-Methyladamant-2-yl)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 10 L-Isoleucine, N-[1-(Adamantan-2-ylcarbamoylmethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(Adamant-1-ylmethylcarbamoylmethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-(2-Methyl-1-(S)-Phenyl-1-Propoxy)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-(2-Methyl-1-(R)-Phenyl-1-Propoxy)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-(4-tert-Butylcyclohexyl)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 15 L-Isoleucine, N-[1-(2-Bicyclo[2.2.1]hept-2-yl)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-(Chroman-2-yl)-2-Oxoethyl)-L-Prolyl] Benzylamide Hydrochloride Salt;  
 L-Isoleucine, N-[1-(2-(Benzofuran-2-yl)-2-Oxoethyl)-L-Prolyl] Benzylamide Hydrochloride Salt;  
 L-Isoleucine, N-[1-(2-(3-Benzoyloxyphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 20 L-Isoleucine, N-[1-(2-(4-Benzoyloxyphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-(2-Benzoyloxyphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-(3-Phenoxyphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-(2-Phenoxyphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-(3,4,5-Triethoxyphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 25 L-Isoleucine, N-[1-(2-(Benzo[1,3]dioxol-5-yl)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-Oxo-2-[4-(2-Phenoxyethoxy)-Phenyl]-Ethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-(4-Phenoxyphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-(2,4,6-Trimethoxyphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-(2,3-Dimethoxyphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 30 L-Isoleucine, N-[1-(2-(2,6-Dimethoxyphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-(1-(4-Methylphenyl)cyclohexyl)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-(1-(4-Chlorophenyl)cyclohexyl)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-(2,3,4-Trimethoxyphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 L-Isoleucine, N-[1-(2-(1-Phenylcyclohexyl)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 35 L-Isoleucine, N-[1-(2-(2,4,5-Trimethoxyphenyl)-2-Oxoethyl)-L-Prolyl] Benzylamide;  
 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] L-Proline Benzyl Ester Hydrochloride;  
 L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] Benzylamide Hydrochloride;  
 L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 2-Phenethylamide Hydrochloride;  
 L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 3-Phenylpropylamide Hydrochloride;  
 40 L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 4-Phenylbutylamide Hydrochloride;  
 L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 2-(Pyrid-2-yl)ethylamide Dihydrochloride;  
 L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 2-(4-aminophenyl)ethylamide Dihydrochloride;  
 L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 3-(4-[N-Carboallyloxy]aminophenyl)propyl Ester Hydrochloride;  
 45 L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 2-Phenyl-2 oxoethylamide;  
 L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] Tetrahydrofurfurylamide;  
 L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] Naphthalen-1-ylmethylamide;  
 L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 2-(4-Sulfamoylphenyl)ethylamide;  
 L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 4-Phenylpiperidenylamide;  
 50 L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 4-Methoxybenzamide Hydrochloride;  
 L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 3-Methoxybenzamide Hydrochloride;  
 L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 2-Methoxybenzamide Hydrochloride;  
 L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] N-Methylphenethylamide Hydrochloride;  
 L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] (S)- $\alpha$ -methylbenzylamide Hydrochloride;  
 55 L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] (R)- $\alpha$ -methylbenzylamide Hydrochloride;  
 L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 1-methyl-3-phenylpropylamide Hydrochloride;  
 L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] Adamant-1-ylmethylamide Hydrochloride;  
 L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 1-(R)-(1-naphthyl)ethylamide Hydrochloride;



- L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] Cyclohexylmethylamide;  
 L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] Diphenylmethylamide Hydrochloride;  
 L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] tert-Butylamide Hydrochloride;  
 L-Proline, 1-2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 1,2-Diphenylethylamide Hydrochloride;  
 5 L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] Cyclohexyl amide Hydrochloride;  
 1-2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] L-Homoproline Benzyl Ester Hydrochloride;  
 L-Homoproline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] Benzylamide Hydrochloride;  
 L-Homoproline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] Adamant-1-ylmethylamide Hydrochloride;  
 L-Homoproline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] tetrahydrofurfurylamide;  
 10 L-Homoproline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 2-(4-Sulfamoylphenyl)ethylamide;  
 L-Homoproline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] (S)- $\alpha$ -methylbenzylamide Hydrochloride;  
 L-Homoproline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] (1-(S)-[2'-(S)-methylpropyl]-3-phenylprop-2-  
 E-enyl)-amide;  
 L-Homoproline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] (1-(S)-[2'-(S)-methylpropyl]-3-phenylpropyl)-  
 15 amide;  
 L-Isoleucine, N-[1-(2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl)-L-Homoprolyl] Benzylamide;  
 L-[Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 2-(4-(N-Acetyl)aminophenyl)ethylamide;  
 L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 2-(4-(N-Benzoyl)aminophenyl)ethylamide;  
 L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 2-(4-(N-carboallyloxy)aminophenyl)ethylamide;  
 20 L-Proline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 2-(4-(N-Carbobenzyloxy)aminophenyl)ethylamide;  
 L-Homoproline, 1-[2-(3,4,5-Trimethoxyphenyl)-2-Oxoethyl] 3-(4-(N-Carboallyloxy)aminophenyl)propyl  
 Ester Hydrochloride;  
 L-Proline, 1-[2-Adamantan-1-yl-2-Oxoethyl] 3-(4-(N-Carboallyloxy) aminophenyl)propyl Ester Hydrochlo-  
 ride; and  
 25 L-Homoproline, 1-[2-Adamant-1-yl-2-Oxoethyl] 3-(4-(N-Carboallyloxy)aminophenyl)propyl Ester Hydro-  
 chloride.

6. A medicament containing at least one compound according to Claim 1.  
 7. Use of the compounds according to Claim 1 for the manufacturing of a composition for the treatment of  
 inflammation.  
 8. A method for making the composition of claim 3, comprising the following steps:

35



40

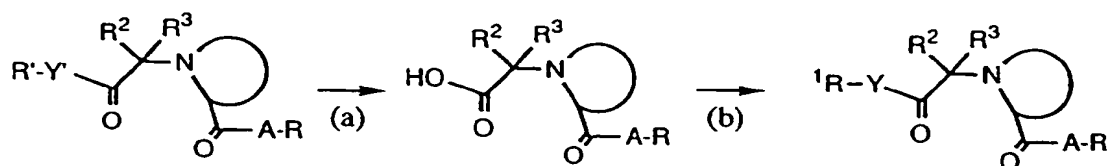
which are

- 45 (a) coupling an N-protected imino acid to an amine or an alcohol to form a C-substituted, N-  
 protected imino acid;  
 (b) removing the protecting group from said C-substituted, N-protected imino acid; and  
 (c) alkylating the resulting imino acid from step (b) at the nitrogen position with an  $\alpha$ -halo ester,  $\alpha$ -  
 halo ketone, or an  $\alpha$ -halo amide.

50

9. A method for making the composition of claim 3, comprising the following steps:

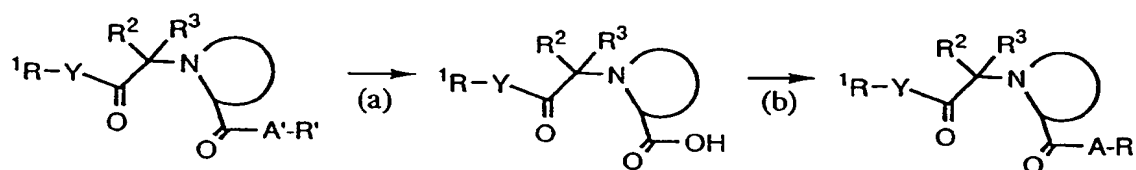
55



which are

- a) deprotecting the 2-position of a first 2-oxoethyl derivative; and  
 b) coupling the resulting acid derivative from step (a) to form a second 2-oxoethyl derivative.

10. A method for making the composition of claim 3, comprising the following steps:



which are

- a) deprotecting the imino acid C-termini of a 2-oxoethyl derivative to form a 2-oxoethyl imino acid;  
 and  
 b) coupling said 2-oxoethyl imino acid resulting from step (a) with an amine or an alcohol to form a C-substituted, 2-oxoethyl imino acid derivative.

(19)



Europäisches Patentamt  
European Patent Office  
Office européen des brevets



(11) Publication number:

**0 564 924 A3**

(12)

**EUROPEAN PATENT APPLICATION**(21) Application number: **93105035.5**(22) Date of filing: **26.03.93**

(51) Int. Cl.<sup>5</sup>: **C07K 5/06**, C07D 207/16,  
C07D 401/12, C07D 405/12,  
C07D 401/06, C07D 211/60,  
A61K 37/02, A61K 31/40,  
A61K 31/44, A61K 31/445

(30) Priority: **08.04.92 US 864998**  
**25.11.92 US 981565**

(43) Date of publication of application:  
**13.10.93 Bulletin 93/41**

(84) Designated Contracting States:  
**AT BE CH DE DK ES FR GB GR IE IT LI LU MC**  
**NL PT SE**

(88) Date of deferred publication of the search report:  
**29.12.93 Bulletin 93/52**

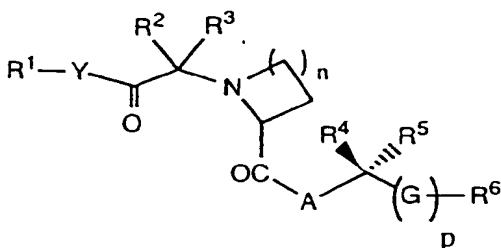
(71) Applicant: **MILES INC.**  
**One Mellon Center**  
**500 Grant Str.**  
**Pittsburgh, PA 15219-2502(US)**

(72) Inventor: **Connell, Richard D.**  
**3 Raynham Road**  
**New Haven, CT 06512(US)**  
Inventor: **Osterman, David D.**  
**715 Manchester Road**  
**Glastonbury, CT 06033(US)**  
Inventor: **Katz, Michael E.**  
**6 Nod Brook Road**  
**Wallingford, CT 06492(US)**

(74) Representative: **Dänner, Klaus, Dr. et al**  
**Bayer AG**  
**Konzernverwaltung RP**  
**Patente Konzern**  
**D-51368 Leverkusen (DE)**

(54) **2-Oxoethyl derivatives as immunosuppressants.**

(57) A class of compounds which suppress human T-lymphocyte proliferation is disclosed. The active compounds essentially contain at least the following structure:

**EP 0 564 924 A3**



European Patent  
Office

## EUROPEAN SEARCH REPORT

Application Number

EP 93 10 5035

### DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
P, X	J. MED. CHEM. vol. 35, no. 23, 13 November 1992, USA pages 4284 - 4296 J. R. HAUSKE ET AL. 'DESIGN AND SYNTHESIS OF NOVEL FKBP INHIBITORS' * the whole document *	1-9	C07K5/06 C07D207/16 C07D401/12 C07D405/12 C07D401/06 C07D211/60 A61K37/02
X	EP-A-0 038 758 (ROQUES, B.; SCHWARTZ, J.-C.; LECOMTE, J.-M.) 28 October 1981 * page 7; examples 49, 59 *	1-3	
X	IL FARMACO ED. SC. vol. 43, no. 12, 1988, pages 989 - 1003 R. CIABATTI ET AL. 'PROLYL DERIVATIVES OF ENALAPRIL AS POTENTIAL ANGIOTENSIN CONVERTING ENZYME INHIBITORS' * page 991; figure III; examples IV-IX * * page 997; table III *	1-3	
A	WO-A-9 200 278 (VERTEX PHARMACEUTICALS INCORPORATED) 9 January 1992		TECHNICAL FIELDS SEARCHED (Int. Cl.5)  C07K C07D A61K
The present search report has been drawn up for all claims			
Place of search MUNICH		Date of completion of the search 13 OCTOBER 1993	Examiner DEFFNER C.
CATEGORY OF CITED DOCUMENTS			
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document  T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons  & : member of the same patent family, corresponding document			

EPO FORM 1501 Q1.12 (P0401)